USE OF BOVINE SOMATOTROPIN (BST) IN MANAGEMENT OF GROWING HEIFERS AND TRANSITION COWS TO IMPROVE GROWTH RATES AND MILK PRODUCTION

By

ALFREDO N. GARCIA GAVIDIA

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1998

To the memory of my parents Alejandro Garcia and Aminta Gavidia de Garcia. To my children Abraham, Manuel and Indira Garcia Mattos and specially to my wife Elsa Mattos de Garcia, who has given to me all the emotional support I have needed to be here working toward this goal.

ACKNOWLEDGMENTS

I would like to thank my professors for all the help I have received from them, especially those who have been members of my Ph.D. committee, Dr. H. Herbert Head as chairman, and Dr. Charles J. Wilcox, Dr. John E. Moore, Dr. Douglas B. Bates and Dr. Ramon C. Littell.

Special recognition goes to Ms. Joyce Hayen, our laboratory chemist, who has given to me all the help I have needed as a son.

I thank Dr. Mary Beth Hall, Dr. Roger West, Dr. William Thatcher and Dr. Charles Staples for their support during this research.

I thank Mary Ellen Hissem and Helen Hester for their friendship.

I thank all the personnel from the DRU, especially Mr. Dale Hissem, James Lindsey, Charlene Roomes, Joe Cline, Robert Tillman, Mary Russell, Helen Lindsey, Elese Griffin and others.

I thank my friends Rafael Román, Diego Rochinotti, John Kobera, Amelia Luengo, Thaís Diaz, Tomás I. Belloso, Andrés Kowalski, Elide Valencia, Carlos Aréchiga, Oswaldo Rosendo, Maria Eugeniia Cadario, Antonio Landaeta, Carlos Vargas, Victor Monterroso, Mario Binelli, Morgan Pletier, David Feitsma, Mehmet Gulay, Alice Moraes, Jennifer Trout and all my classmates who have shared with me during all this time. Finally, I acknowledge all those persons who, in one way or another, have helped me.

TABLE OF CONTENTS

ACKNOWLEDGMENTS iii

ABSTRACT vii

CHAPTERS

1 INTRODUCTION 1

2 MILK PRODUCTION OF FIRST CALF HEIFERS RAISED ON TWO PLANES OF NUTRITION AND INJECTED WITH BST 7

Introduction 7
Body Growth, Puberty and Mammary Gland Development 9
Growth Rates, Age at Calving and Milk Yield 13
Somatotropin, Growth and Mammary Gland Development 18
Materials and Methods 23
Experiment I 24
Animals 24
Experimental design 24
Injections of bST 25
Feeding 25
Animal measurements 26
Statistical analyses 28
Experiment II 29
Animals 29
Experimental design 30
Injections of bST 31
Feeding 31
Animal measurements 32
Milking and sampling 34
Statistical analyses 34
Results and Discussion 35
Conclusions 63

3 EFFECT OF NUTRITIONAL STATUS AND PREPARTUM AND POSTPARTUM BST TREATMENTS ON DRY MATTER INTAKE, ENERGY STATUS, BODY WEIGHT CHANGE AND MILK YIELD OF DAIRY COWS DURING EARLY LACTATION 64

Introduction 64
Dry Matter and Energy Intake During the Transition Period 65
Diet and Intake the During Transition Period 68
Body Condition Score and Transition Period 75
Energy Status and Metabolism During the Transition Period 79
Mechanism of Metabolic Regulation During the Transition Period 83
Materials and Methods 88
Experimental Animals 88
Injections of bST 93
Feeding Program 94
Data Collection 94
Feed intake 94
Body weight and body condition score 95
Milk samples 95
Blood samples 95
Net energy intake and energy status 96
Statistical Analyses 97
Results and Discussion 98
Dry Matter Intake, Energy Status, and Changes in Body Weight and Body Condition 103
Milk Yield, 4% FCM and Milk Constituents 123
Conclusions 134

4 EFFECTS OF NUTRITIONAL STATUS AND PREPARTUM AND POSTPARTUM BST TREATMENT ON METABOLIC HORMONES AND OVARIAN ACTIVITY OF DAIRY COWS DURING EARLY LACTATION 136

Introduction 136
Insulin, Somatotropin and Insulin-like Growth Factor-I 137
Somatotropin, IGF-I and Ovarian Activity 143
Other Metabolic Hormones 147
Materials and Methods 149
Insulin Assay 151
Prolactin Assay 154
Insulin-like Growth Factor I (IGF-I) Extraction and Assay 157
Somatotropin Assay 161
Triiodothyronine Assay 164

Thyroxine Assay 165
Statistical Analyses 167
Results and Discussion 168
Conclusions 189

5 GENERAL CONCLUSIONS 190

APPENDIX 194

REFERENCES 198

BIOGRAPHICAL SKETCH 222

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree Doctor of Philosophy

USE OF BOVINE SOMATOTROPIN (BST) IN MANAGEMENT OF GROWING HEIFERS AND TRANSITION COWS TO IMPROVE GROWTH RATES AND MILK PRODUCTION

By

Alfredo N. Garcia Gavidia

May 1998

Chairperson: H. Herbert Head
Major Department: Dairy and Poultry Sciences

Milk production of 194 Holstein heifers was evaluated for effects of plane of nutrition during the growth period from 90 to 365 d and bST injections (12.36 mg/d or 500 mg/14 d) from 220 to 365 d of age. Two groups of Holstein heifers were utilized (I = 63 ; II =183 heifers). Heifers were fed either alfalfa silage or corn silage plus grain concentrates. Actual or 305-d and total milk yield records were used in the analyses. Models included bST, diet, season of calving, year of calving, age at calving, calving weight, DIM, DO ADG, and appropriate interactions. The ADG from 220 to 365 d , time period when bST was injected, was significantly and positively associated with the milk yield ($P \leq 0.0208$). Effects of DIM, DO and season of calving were significant. The mean yields were 6841 (n= 52) and 7024 (n= 56) kg/305-d for heifers on control and high

energy diets without bST ; 7006 (n= 36) and 7076 (n= 27) kg/305-d for heifers fed control and high energy diets plus 35.7 mg bST/d; and 6706 (n= 9) and 6860 (n= 14) kg/305-d for heifers on control and high energy diets plus 12.6 mg bST/d. Heifers raised on different planes of nutrition injected or not with bST during the peripubertal period produced the same amount of milk during the first lactation. The second study included 48 Holstein cows to evaluate prepartum and postpartum treatments of bST and nutrition status on DMI, energy status, milk yield, some metabolic hormones and progesterone (P_4) during early postpartum period. Treatments were arranged in a 2x2x2 factorial and included prepartum injections of bST (0 and 5 mg/d), postpartum injections of bST (0 and 5 mg/d), and diet (0 and 15 % WCS). Models included the main effect of treatments and the two- and three-way interactions of treatments. Prepartum bST treatment resulted in higher concentrations of ST, IGF-I and glucose before parturition. Prepartum and postpartum bST treatment resulted in increased DMI ($P < 0.0281$), slightly higher milk yield, improved energy status ($P < 0.1038$) and increased concentrations of ST and IGF-I during early postpartum period.

CHAPTER 1
INTRODUCTION

One of the first biotechnology derived products available for use in animal production is Bovine Somatotropin (bST). In contrast to steroids, bST is a protein hormone. Many investigations have demonstrated milk yield and lactation persistency responses to bST for all breeds of dairy animals examined. However, quality of management apparently is a major factor affecting magnitude of milk response to bST. Productive efficiency of dairy cows has been defined as the yield of milk and milk components relative to the nutritional cost of maintenance, lactation, and the return of the cow to the level of body condition that existed before onset of the lactation.

On the other hand, dairy farmers must invest capital, time and feed in order to raise replacements for their dairy herds. Raising heifer replacements will increase profitability when it can be accomplished more efficiently with reduced costs. One way to decrease the cost of raising replacements is by decreasing the time for them to reach first parturition. A major factor that affects age at first calving is the limited capacity of heifers to partition nutrients when attempts are made to increase their growth rates. Possible effects of this limited capacity are on development of mammary epithelial tissue or accretion of body protein, both of which are important but remain secondary to partitioning of nutrients for more rapid growth (Hoffman and Funk, 1992).

The heifer raising program may be a separate enterprise on a dairy farm but it is likely that it would be an integral part of dairy operations. Advanced age at first parturition usually is considered to be detrimental for dairy profit because of increased labor and feed costs and delayed income from sale of milk (Lin et al., 1988). It has been recommended that replacement dairy heifers reach first parturition at about 22- 24 mo of age and at least 550 to 560 kg of live weight after calving (NRC, 1989; VandeHaar, 1998). These recommendations are largely based upon the positive correlations between size at first calving and milk production.

Growth of dairy heifers has been described as a natural expansion process such that the heifer increases in external size in all directions at a rather uniform but not constant rate (Brody, 1945). Growth of dairy heifers is affected by many factors including genetics, nutrition, health and management. Maturation of the reproductive system occurs along with an increase in body weight and size (Brody, 1945). Traditionally, for cattle, weight has been used as the measurement unit that is representative of animal size. However, skeletal measurements in conjunction with body weight may be better indicators of an animal's size (Heinrichs and Hargrove, 1987, 1991; Swanson et al., 1967).

Many years of research have resulted in recommendations for growth of dairy heifers during the prepubertal and postpubertal growth periods. Because research has shown the economic benefits of first parturition between 22-24 mo of age, it is necessary to evaluate various alternatives of feeding and management of dairy heifers that will allow the farmer to achieve these target body weights at first calving. Several

management schemes have been proposed to alter heifer growth rates during different growth periods and to increase body size (weight and height) of heifers at time of first calving without affecting subsequent milk production. Reducing average daily gain (ADG) during the prepubertal growth period followed by increased growth rates after puberty requires that changes in energy intake of heifers be made to accommodate these growth rates. This can be accomplished by changing energy concentration of the diet or quantities of nutrients fed during both prepubertal and postpubertal periods (Park et al., 1987). Changes in quality and quantity of dietary protein fed also will lead to modifications in heifer growth rates and may improve feed conversion and efficiency (Tomlinson et al., 1997). Use of partitioning agents such as β-agonists or use of growth regulators such as somatotropin (ST) and/or steroids may be useful for altering utilization of nutrients by growing heifers and in this way affect growth, as measured by weight and height (Hoffman and Funk, 1992).

Many studies have demonstrated that when ST was used in animals during growth periods, nitrogen retention, live weight gain and lean content of the carcass were increased. Researchers have reported that exogenous ST increased ADGs and significantly altered the normal allometric pattern of tissue growth. This resulted in increased rates of skeletal muscle growth (protein deposition), and reduced rates of adipose tissue deposition. Thus, based on results that described improved growth performance and body composition when bST was used, it is important to consider whether bST could be used as a positive growth regulator in heifer rearing programs on commercial dairy farms.

Because increased rate of skeletal growth and reduced adipose tissue deposition of dairy heifers typically occurs during the prepubertal growth period, use of bST may be beneficial; especially if bST could be used after puberty or during the pregnancy growth periods. This could have beneficial effects on milk production during first lactation. Growth promoting and repartitioning effects of exogenous bST already have been demonstrated; thus, manipulation of growth by use of repartitioning agents should be evaluated critically.

On the other hand, farmers have the objective to keep cows producing milk throughout the year. Therefore, replenishment of their body condition early after parturition is of critical importance. Plane of nutrition during lactation can have a significant effect on milk yield and on timing of the onset of normal ovarian activity postpartum. Repletion and/or depletion of body energy of lactating cows may be quantified as a tool to determine energy balance and tissue mobilization. Repletion-depletion of body energy has been measured qualitatively as body condition score (BCS). Values for BCS may be used to determine the extent of dietary energy deficiencies during weight loss in early lactation and to estimate the feed required to regain body weight in later stages of lactation.

Lactation results in a decrease in the rate of fatty acid synthesis in adipose tissue while at the same time causing an increase in the mobilization of body tissues, especially during early lactation. These adaptations occur to facilitate the preferential use of lipogenic precursors by the mammary gland. Hormones responsible for these adaptations were shown to be Insulin (INS), Prolactin (PRL), and ST. Nutritional status modifies

serum concentrations of ST; metabolites such as glucose, non-esterified fatty acids (NEFA), and amino acids also have been postulated to be secondary factors that modulate ST secretion. Collectively, data demonstrated that under certain physiological conditions such as lactation, alterations in energy metabolism may influence hypothalamic-pituitary function. ST is a homeorhetic controller that shifts the partitioning of nutrients, and thus is involved in coordination of the metabolism of various organs and tissues. Changes in tissue metabolism involve both direct effects on some tissues and indirect effects mediated by ST-dependent somatomedins (IGFs) for other tissues (Bauman,1992).

Increased voluntary intake has been seen in bST-supplemented cows. Increased voluntary intake usually occurs a few weeks after initiation of bST-supplementation and persists during the time bST is supplemented. However, the magnitude of dry matter intake)DMI) increase depended on the response in milk yield and the energy density of the diet. Because cows in early lactation have a negative energy balance, and because of the negative correlation between energy balance and concentrations of circulating IGF-I during early lactation and of the role of IGF-I in steroidogenesis, IGF-I has been described as a mediator of energy balance that affects reproduction. It is likely that the relationships among bST, IGF-I and reproductive efficiency would be sensitive to the nutrient and energy status of the animal.

An increased BCS is directly associated with positive energy balance and an increase in energy balance is associated with an increase in circulating concentrations of IGF-I during early lactation, and thus, with an increased production of progesterone (P_4) by the ovary during subsequent estrous cycles. Conversely, an increase in milk yield

during early lactation is associated with decreased concentrations of IGF-I in plasma and reduced energy balance. Thus, goals for dairy farmers should be oriented to maintain the DMI of the cows during the transition period before parturition, and to increase the DMI of the cows as soon as possible during early lactation. This could be one way to improve the recovery of the energy balance during the early postpartum period and to maintain elevated concentrations of IGF-I in plasma. One expected result would be recuperation of BCS at a faster speed and as a result it is likely that P_4 secretion would increase and ovarian activity would increase. This may be a useful way to improve reproductive efficiency by shortening the interval between calving and first estrus and probably the calving interval (CI).

Two bST studies were conducted at the Dairy Research Unit (DRU) of the University of Florida. Main objectives for these research studies were two-fold. First was to evaluate effects of bST on growth rates and subsequent milk yield of Holstein heifers raised on two planes of nutrition. Second was to evaluate the effects of prepartum and postpartum bST treatment and nutritional status on feed intake, energy status, milk yield, some important metabolic hormones and reproductive response during the early postpartum period of high producing Holstein cows.

CHAPTER 2
MILK PRODUCTION OF FIRST CALF HEIFERS RAISED ON TWO PLANES OF NUTRITION AND INJECTED WITH BST

Introduction

Dairy farmers invest money, labor and feed to raise replacements for their dairy herds. Growth of dairy heifers is affected by genetics, nutrition, health, environment and management, among others. However, the rate of gain achieved often is affected by economics of the heifer raising enterprise on the dairy farm, or when dairy heifers are raised by professionals and then returned to the dairy. The cost to raise a dairy heifer to first calving recently has been estimated to be $1200 (VandeHaar, 1998) but it clearly will differ across locations and management systems. Because of this high estimated cost more rapid growth rates of heifers have been recommended in order to reduce the time to reach first calving and, therefore presumably to reduce the costs expended to raise them to first calving. However, even though heifers may be raised at more rapid rates the dollar savings may be less than anticipated because so many of the costs are fixed. Moreover, if the production of the heifers during first and subsequent lactations is less than it should have been, because of reduced mammary development, as has been reported by Sejrsen and Purup (1997), then the savings made in raising them rapidly will

largely be offset and, in the worse case, the income producing ability of these rapidly raised heifers may be much less than that of heifers that calve at older ages and/or at lighter body weights (Van Amburgh and Galton, 1994; VandeHaar, 1998).

Growth rates of heifers should be based on body weight gain and should coincide with the parameters of desired skeletal growth (Sejrsen et al., 1982). Management programs used to raise replacement heifers need to be assessed to assure that the maximum attainable milk production potential of each heifer results when she enters the producing herd. Recommendations for raising dairy heifer replacements currently are that they should first calve at about 22-24 mo of age and with a post-calving body weight of 550-560 kg (NRC, 1989; VandeHaar, 1998; Waldo, 1987). These growth rates are possible as long as no limitations exist such as diseases, other health problems, or poor management practices at critical stages of growth. These recommendations for age and target weights have arisen from the often observed positive association between size at first calving and milk production. Growth rates utilized need to minimize the negative effect, if any exists, that accelerated growth rates may have on milk production. Desired final body weights can be achieved without necessitating extremely rapid growth rates during the critical peripubertal growth period. Thus, the overall goal is to raise heifers to reach adequate body sizes at puberty, at first breeding, and at first calving with well-developed mammary glands so they enter the producing herd fully capable of expressing their genetic potential.

Body Growth, Puberty and Mammary Gland Development

Large dairy breeds such as Holsteins usually reach puberty between 9-11 mo of age and at an average body weight of 250-280 kg; this is at about 40-50% of their mature body weight. Level of feeding of replacement heifers is the main source of variation associated with growth rates within a breed (Moran and Roche, et al., 1989; Schillo et al., 1992). Method of raising heifers can affect their lactation performance because extremes of growth rate are detrimental to milk production during the lactations that follow. Heifers that grow at slower than desired or recommended rates (NRC, 1989), because they are fed at too low a plane of nutrition, will reach puberty at older ages. Therefore, if they still are to calve at about 22-24 mo of age they must be bred at lighter body weights. The alternative is to wait until they have reached satisfactory body weights at older ages before the are bred and thereby calve at older age. Breeding heifers at lighter body weights than recommended results in smaller body size at calving, less well-developed mammary glands, and a greater occurrence of dystocia at calving (Hoffman and Funk, 1992). Therefore, these heifers are expected to have lower milk production during first and second lactations.

Potential for milk production is a direct function of the number of secretory cells in the mammary glands and their milk synthesizing activity during lactation (Forsyth, 1996; Knight and Wilde, 1987), with cell number probably being the limiting factor (McGrath, 1987). In addition, it is important that lactating animals have the capacity to eat enough of the correct nutrients and are able to partition these nutrients to the

mammary gland and other important organs to ensure high levels of milk production. For these and other reasons related to economics and management, the growth rates of replacement heifers is of great importance for their subsequent performance in the dairy herd.

Growth and development of mammary cells occurs in distinct phases that are related to reproductive development during fetal life, around the time of puberty, during pregnancy, and in preparation for and during lactation. Mammary growth is dependent upon mammogenic hormones such as ST, estrogen (E_2) and PRL, and various growth factors (Forsyth, 1996; Tucker, 1981, 1987). Most mammary gland development occurs during pregnancy. The extent of mammary development during puberty is, both qualitatively and quantitatively, less pronounced than during pregnancy. However, pubertal mammary development is of great importance for the future milk producing ability of the dairy heifer and cow (Foldager and Sejrsen, 1987; Johnson, 1988; Sejrsen, 1994; Sejrsen and Purup, 1997). Amount and type of growth of the mammary system that takes place throughout various postnatal growth periods can affect how much milk the heifer will produce during the lactations that follow.

From birth to 3 mo of age, and during the postpubertal period (12 mo of age through the first third of pregnancy), the mammary glands of heifers develop at essentially the same rate as the rest of the body. This growth is termed isometric growth and it is at this time that the mammary fat pad, vascular system and extra-parenchymal tissues increase substantially, but the epithelial components (small ducts) grow only at the rate the rest of the body is growing. However, between these two isometric growth

periods (from 3 - 9 or 10 mo of age) and from 3 mo of pregnancy through the time of calving, but especially during the last trimester of first pregnancy, mammary growth is two to four times faster than the rest of the body is growing. This is termed allometric growth and during this period the mammary fat pad and vascular and supportive systems continue to increase and the ductal epithelium of the parenchyma expands into the fat pad (Sinha and Tucker, 1969). Once heifers have been bred and become pregnant, the mammary glands are exposed to the hormones of pregnancy plus various growth factors which stimulate the second allometric growth period seen most clearly during the last trimester of pregnancy.

During the prepubertal period two factors known to affect the contents of parenchymal tissue that is laid down in mammary glands are plane of nutrition and peripheral concentrations of ST (Sejrsen et al., 1989). It has been reported that the hormonal shift that controls changes in expansion of ductal epithelium into the fat pad may be mediated, in part, by the mammary fat pad itself. The fat pad may receive hormonal signals which act on the fat cell, which in turn responds by sending appropriate chemical signals to the ductal tissue. During the allometric phase of prepubertal mammary development, rates of parenchymal tissue growth is 1.8-3.5 times greater than rates of growth observed in the whole body (Tucker, 1981). It has been suggested that hormonal changes at puberty are the signal for returning mammary glands to isometric growth, but the exact signal has not been identified at this time (Forsyth, 1996; Van Amburgh and Galton, 1994).

Mammary gland parenchyma was affected adversely in heifers that had high ADGs from 175 to 320 kg of body weight (BW) (Sejrsen et al., 1986). Greater concentrations of DNA and RNA in mammary dry fat-free tissue (DFFT) have been observed in heifers fed a low plane of nutrition before and after 200 kg BW (Niezen et al., 1996). Moreover, *ad libitum* feeding of rations before onset of puberty caused a 23% reduction in epithelial tissue weight and a 32% reduction in DNA content of the mammary glands. Therefore, body weight of 90 kg was considered the critical weight at which high growth rates (ADGs) began to affect subsequent milk yield (Foldager and Sejrsen, 1987). However, the deleterious effect of rapid growth rates on mammogenesis during the prepubertal period due to excess body fat deposition did not cause a decline in subsequent milk production during first lactation (Capuco et al., 1995). The range of ADGs that appeared to cause a detrimental effect seems to have an upper limit ADG of 0.91 kg/d or greater during the allometric growth phase. This was concluded from evaluation of published research on mammary parenchyma growth from 11 different studies (VandeHaar, 1998). In these studies, selected to evaluate because heifer growth rates were greater than 0.91 kg/d (high plane of nutrition), the high ADGs during the allometric growth period caused mammary parenchymal growth differences that were between +3 to -62% of the parenchymal growth in the control heifers raised at recommended growth rates (NRC, 1989). Based upon these findings VandeHaar (1998) suggested that there was good evidence that growth rates of heifers during the allometric growth phase can be much greater than currently recommended if the diet fed to the heifers is well-balanced and has sufficient protein content.

Although growth rates during both the prepubertal and postpubertal periods have significant effects on calving weights, only growth rates during the postpubertal had a significant effect on milk yield (Hoffman and Funk, 1991). Waldo et al. (1988) considered that absolute growth of mammary parenchyma may be enhanced by rapid growth after puberty or during pregnancy. However, it is not certain that this explanation is needed to account for the postpartum growth rate effect. Milk yields during first lactation were more positively correlated with age than with body weight (Moore et al., 1991). Heinrichs and Hargrove (1987) observed that dairy herds with heavier replacement heifers had greater milk yields, implying these heifers also produced well and rapid growth rates were not detrimental. They also established that average herd production was correlated with estimated weight of contemporary Holstein heifers at 24 mo of age (0.34) and also with estimated height at withers (0.41). Additionally, there was a benefit in milk yield when Holstein heifers calved at body weights of 590-635 kg (Keown and Everett, 1986).

Growth Rates, Age at Calving and Milk Yield

It is possible that high milk production can be attained when heifers are large and calve at 22-24 mo of age. Current recommendations for Holstein heifers (NRC, 1989) are for ADGs of 0.6 to 0.8 kg/d. Clearly, the lower recommendation in this range will not result in growth rates that will allow heifers to calve at 22-24 mo and at an adequate body weight and body size. An ADG of at least 0.8 kg /d, or greater, will allow heifers to reach desired weights at desired ages for a 24 mo calving age but not earlier, if we assume that

the birth weight is 40 kg, growth rates during the first 2-3 months of age are at least 0.45 kg/d and subsequent real growth rates, excluding weight of conceptus up to calving, are 0.8 kg/d or greater. Clearly, if heifers only have ADGs of about 0.45 kg/d during the first 2-3 mo of age and we consider the growth rates (body weight change) associated with conceptus development and the expected 45-68 kg loss in weight due to calving, then ADGs during the peripubertal period, the postpubertal period, and also during pregnancy must be even greater. Thus, it is possible to calculate that Holstein heifers that reach puberty by 8-11 mo of age and at 40 to 50% of mature body weight can be bred at 13 mo when they are about 50 to 60% of mature body size. If they conceive at or before 15 mo of age then they will calve at about 24 mo, and possibly earlier, and it will be possible for them to achieve the 550-560 kg post-calving target weight. Interestingly, heifer raising programs of the high producing herds in Wisconsin had heifers reach 360 kg of body weight at 13 mo and a precalving body weight of 600 kg at 24 mo (Hoffman and Funk, 1992). Moreover, analyses of milk production from commercial herds in Pennsylvania indicated that heifers from high producing herds were heavier at 24 mo than those of low producing herds (Heinrichs and Hargrove, 1987). These authors found similar trends for Guernseys, Jerseys, Brown Swiss, Ayrshire and Milking Shorthorns (Heinrichs and Hargrove, 1991; 1994). However, in some cases, heavier heifers at first calving produced less milk, protein and fat than did the lighter heifers. Heavier cows possess little, if any, superiority in feed efficiency over smaller cows, but there is a positive relationship between body size at first calving and the first lactation milk yield. Maximal milk production during first lactation was found when heifers weighed between 600-650 kg at

calving (Keown, 1986). Results from these analyses indicated that age was less important than body weight.

ADGs of 1.0 kg/d or greater for heifers have been recommended by many authors (Gardner et al., 1977; Kertz et al., 1987; Park et al., 1987; Wright and Russell, 1991). However, when growth rates exceeded 1.0 kg/d during the first 12 mo of life this appeared to be associated with reduced milk production potential, presumably because of a significant reduction in mammary secretory tissue laid down at this time. Data suggested that accelerated prepubertal gain had less effect on milk yield when calving ages were greater than 23 mo. Therefore, the negative effect of accelerated prepubertal growth appeared to be coupled with early breeding and calving and this suggested that other biological mechanisms may play a role in the reduction in milk yield when age at calving is less than 22 mo due to the accelerated growth rate of replacement heifers (Hoffman et al., 1996). Nonetheless, heifers calving at an average age of 22.9 mo produced more total milk per day of age than those calving at 26 mo (Lin et al., 1986). Daccarett et al. (1993) reported that feeding 124 % of NRC (1988) requirements from 3-6 mo and 115 % of requirements from 6-24 mo of age resulted in heifers with greater skeletal development and body weight without excessive fattening and with potential for earlier calving.

Physiological maturity of heifers is important at time of breeding, especially with the consideration to the qualitative and qualitative development of the mammary glands. Achieving earlier calving by breeding at lighter body weights may not allow for adequate mammary gland growth, which in turn may be expected to impair milk yield of heifers

during first lactation. Nevertheless, moderate growth rates of 0.57 to 0.76 kg/d during the first 12 mo, followed by more rapid growth rates during pregnancy, did not impair mammary tissue development (Harrison et al., 1983; Sejrsen et al., 1982; USDA/DHIA, 1990). Weight of heifers at first calving relative to mature size may be associated with the negative effect on milk production. To optimize milk production during first lactation, body weight of heifers at calving should be at least 82% of their expected mature weight. This will limit the need of smaller first calf heifers to compete with larger heifers and cows, if not grouped separately, for feed and better ensure adequate DMI. It is known that heifers with smaller body size at calving had decreased DMI and also preferentially used energy consumed for growth (Van Amburgh and Galton, 1994). Smaller heifers compensated for their smaller size by directing greater proportion of ingested nutrients to growth processes during the first lactation and therefore, milk yield was less even though their persistency of lactation may have been greater.

It seems clear that energy allowance provided heifers before they reached maturity influenced subsequent milk production during first lactation. Feeding levels that resulted in daily gains of large dairy breeds above 0.6-0.7 kg during the peripubertal period had a negative effect on growth of the mammary parenchyma (Foldager and Sejrsen, 1982). Heifers fed at 85% of NRC (1988) recommendations for energy (ADG of 0.7 kg) from 6 to 10 mo of age and then fed a high energy high protein diet through 12 mo of age produced more milk during the first 250 d of lactation than heifers fed a diet corresponding to 90-100% of NRC (1988) recommendations, or heifers fed a high energy high protein diet *ad libitum* during 6 to 12 mo of age (Peri et al., 1993). However, other

experiments have indicated that mammary growth is unaffected by the energy concentration of the feed (Head et al., 1991; Van Amburgh and Galton, 1994; Waldo et al., 1988). Moreover, VandeHaar (1998) suggested that high protein levels may prevent the negative effect of overall feeding level. It has been reported that the negative effect of high feeding level was unaffected by the amount of by-pass protein fed to the heifers. Similar results were observed by Van Amburgh and Galton (1994). Recent evaluation of literature related to mammary development and diet by VandeHaar (1998) suggested that the ratio of crude protein (CP) intake to energy intake during the peripubertal allometric growth period was a determinant of the negative effect on mammary parenchyma growth. This interpretation was based upon feeding level across 11 experiments reported in the literature. Although diets commonly are evaluated based upon their protein content/unit of dry matter (DM), heifers actually need a specific percentage of the calories in their diet to come from protein. The amount of CP recommended by the NRC (1989) is 60g CP/Mcal of energy for heifers from 3-6 mo of age and 50 g CP/Mcal of energy for heifers from 6-12 mo of age. VandeHaar (1998) reported from analysis of data reported in 11 studies that mammary development was negatively correlated ($r=-0.78$, $P<0.01$) with the protein to energy ratio (g CP/Mcal of ME). In contrast, he found that the actual rate of gain of the heifers that had ADGs greater than 0.91 kg/d in the 11 trials was negatively but not significantly correlated with mammary parenchyma development. He concluded that inadequate protein in the diet of rapidly growing heifers may have been responsible for the impaired development of the mammary parenchyma. Importantly, in the study of Capuco et al. (1995), mammary development only was impaired when the diet they fed to

raise heifers at a rapid rate was based on corn silage and contained 54g CP/Mcal of energy; whereas, no impaired development of mammary parenchyma occurred when the diet was based on alfalfa silage and contained 83g CP/Mcal of energy. This suggested that the low protein content of the corn silage-based diet probably was the reason for the impairment. Support for the conclusion that CP may be limiting in high energy diets was provided when VandeHaar (1998) used the CP/ME (metabolizable energy) ratio alone in the analysis of heifer growth and mammary growth data from the 11 studies; it accounted for 75% of the variation in reported mammary growth responses. Unfortunately, very few research studies with growing heifers have evaluated effects of dietary protein on mammary development and few have critically evaluated the relative amounts of by-pass and rumen degradable protein and associated effects during the peripubertal period.

Somatotropin, Growth and Mammary Gland Development

In animal production systems growth is a long-term process and many organs show large changes in their mass throughout life; these organs change because of the physical or metabolic work that each performs during the growth process and that they will perform during adult life. Changes in the relative tissue distribution of growing replacement heifers may be a function of genetic selection for milk yield over time (Hoffman and Funk, 1992). Mechanisms responsible for hyperplasia or hypertrophy, or both, that are responsible for increases in organ and overall body mass, generally reflect those in effect during developmental growth (Reeds and Fiorotto, 1990). The composition of the body changes with maturity. Thus, with increasing metabolic size,

rates of protein accretion shift to become a greater proportion than the fat deposition but protein turnover continues to increase (Fox and Black, 1984). DiMarco et al. (1987) suggested that the increase in body mass occurred in three phases. These increases were during the early post-weaning period when growth was hypertrophic, during the prepubertal stage (200-300 kg BW) when both hyperplasia and hypertrophy were occurring, and finally, when animals were greater than 350 kg of (BW)and hyperplasia was the predominant type of growth. Therefore, weight alone is not a perfect measure to describe growth. For example, excess accumulation of lipids vs. lean tissue can occur in fast-growing heifers. Thus, increases in body weight alone can be misleading and to measure growth it should be coupled with other physical measures such as height at withers, body length, width at hooks and pins, or others.

The endocrine system and many growth factors control body growth. Body weight gains and amount of ST/g of anterior pituitary tissue were positively correlated (Armstrong and Hansel, 1956). Somatotropin is considered to be anabolic, anti-INS and lipolytic (Bass and Gluckman, 1990). Heifers from high genetic groups may have different growth characteristics, different concentrations of circulating ST, and the ratio of height to weight for these heifers was higher. Thus, a unit of gain may be represented by different ratios of fat, protein and ash (Bonczek et al., 1988). In short-term growth studies, ST markedly altered nutrient utilization resulting in improved growth rates, feed conversion, and increased nitrogen retention in sheep (Wallace and Bassett, 1966; Wynn et al., 1979) and steers (Moseley et al., 1982). Long-term experiments with cattle, sheep and pigs showed that ST increased ADGs (Brumby, 1959; Machlin, 1972; Turman and

Andrews, 1955; Wagner and Veenhuizen, 1978), significantly altered the normal allometric pattern of tissue growth, and this resulted in increased rates of skeletal muscle growth (protein deposition) and reduced rates of adipose tissue deposition (Machlin, 1972; Muir et al., 1983; Wagner and Veehuizen, 1978). Prepubertal Holstein heifers fed on pastures and treated with exogenous ST for 21 wk had greater ADGs than uninjected control heifers (Sandles and Peel, 1987). Injections of bST increased ADGs of heifers 17-295% during the period from 1.5-18 mo of age (Early et al., 1990; Enright, 1989; Grings et al, 1990). In other studies results of exogenous ST injections on growth rates and body composition were inconclusive (Murphy et al., 1991). However, Stelwagen et al. (1992) reported that injecting pregnant heifers with 20 mg bST/d resulted in greater weight gains during the last trimester of gestation than injecting 40 mg bST/d.

Somatotropin is required for maximum pubertal growth and for development of mammary glands (Sejrsen et al., 1986). However, serum concentrations of ST are reduced in prepubertal (3.5-8 mo) heifers fed a high plane of nutrition, and in pubertal (8-12 mo) dairy heifers (Capuco et al., 1995; Sejrsen et al., 1982). Exogenous administration of ST increased the amount of mammary parenchyma (Sandles et al., 1987; Sejrsen et al., 1986). Although ST may have an important role in mammogenesis, when beef heifers were treated with ST the growth rates were enhanced but the ST treatment did not overcome the deleterious effect of high energy diet on subsequent milk production; this suggested a deleterious effect on mammary growth (Buskirk et al., 1996). However, Hall et al. (1992) reported that bST treatment of beef heifers overcame the negative effect of high level of feeding on calf weaning weights over three parities.

Moreover, primiparous cows treated with bST from d 118 of lactation had increased milk yield, yield of milk components and metabolic activity (RNA) of mammary tissue, but no increase in mammary cell numbers (DNA) was detected and there was no increase in Plasmin in milk (Binelli et al., 1995). The injection of bST to enhance lactation caused a reduction in milk Plasmin levels. This change in Plasmin due to bST during lactation implicated bST as the inhibitor of the gradual involution of mammary tissue that typically occurred during ongoing lactation (Politis and Turner, 1990). The yield of 4% FCM following prepartum bST treatment was 19% higher in the heifers treated with 20 mg bST/d; however, no increase in milk yield of Holstein heifers occurred during first lactation when the heifers received 40-mg bST/d (Stelwagen et al., 1992). No positive effects of bST injections were obtained when heifers were injected during the breeding period (13-18 mo of age) (Grings et al., 1990) or when bST-treatment was administered from 7-11 mo of age (Murphy et al., 1991).

Although no direct cause and effect relationship between circulating concentrations of ST and mammary function during lactation has been detected, there does appear to be an association of bST with mammary growth. ST is the principal controller of mammary tissue growth during the prepubertal growth period, and was responsible for enhanced mammogenesis in heifers treated with 20 mg bST/d prepartum (Stelwagen et al, 1992) This supports the hypothesis that the negative effect of feeding high levels of energy on pubertal mammary growth likely was mediated by reduced secretion of ST (Sejrsen, 1994). Circulating concentrations of IGF-I are increased in heifers fed high energy levels (Breier et al., 1988) and ST seems to act on the mammary

gland of prepubertal heifers indirectly via IGF-I. This argues against the conclusion that the detrimental effect of feeding level on lactation potential is mediated directly on the mammary gland by ST. However, the true relationship between ST, IGF-I and mammary growth may be modified by IGF-I binding proteins found in blood and tissues and also by the local production of IGF-I in the mammary gland (Sejrsen, 1994). IGF-I is increased by exogenous administration of ST (McShane et al., 1989; Crooker et al., 1990) and can stimulate multiple cellular responses related to tissue growth, including synthesis of DNA and cellular proteins (Clemmons and Underwood, 1991; Purup et al., 1995).

Receptors for IGF-I have been demonstrated in mammary tissue of cows; these receptors are involved in the IGF-I-mediated actions of ST in the mammary gland (Dehoff et al., 1988). Moreover, mammary gland IGF receptors change with the physiological state of the tissue. For example, IGF-I receptor numbers decline during the prepartum period, increase up to 75% during lactogenesis and this increase is associated with increased binding of IGF-I to mammary microsomes. Finally, receptors decline during the postpartum period (Dehoff et al., 1988). This supports the theory that IGF-I plays an important role in modulating the metabolic activity of bovine mammary gland by acting directly on normal mammary epithelial cells to stimulate DNA synthesis and to increase cell numbers. However, Winder et al. (1992) reported that mammary growth during pregnancy was not regulated at level of the Type-1 IGF receptor nor was it associated with increased binding of IGF-I to mammary microsomes (Dehoff et al., 1988).

It is clear that additional research is needed in order to describe the relationships between mammary gland growth, skeletal growth, body weight increases, and subsequent milk yield during first lactation and how these are affected by plane of nutrition, especially during the critical peripubertal allometric growth phase. Moreover, changes in nutrient requirements of heifers to support growth at different ages also will be important to determine. This will be a very important way to establish the most desirable growth rates during the various time periods through breeding and time of first calving. This will be the most satisfactory way to formulate satisfactory diets and management practices to permit maximal growth potential of heifers and have them reach target weights at early ages so they can enter the producing herd at younger ages.

Objectives of this research were to describe the relationship between two planes of nutrition during the peripubertal growth period that were intended to support normal and high growth rates (ADGs) of Holstein heifers and the use bST injections to increase circulating concentrations of ST during the peripubertal growth period. Injections of bST were either 12.6 or 35.7 mg bST/d during this period. A major objective was to quantify actual growth rate of heifers, use of bST, the interaction of nutrition and bST, if any existed, and the association with the first lactation milk yields of these heifers.

Materials and Methods

Two hundred forty-eight Holstein heifers were assigned to two experiments conducted to evaluate growth rates of heifers fed different planes of nutrition and treated or not with recombinant bST. Trials were conducted at the Dairy Research Unit of the

University of Florida. The experiments were conducted as separate trials over a 3 yr period and had different experimental designs, feeding programs and bST regimens; therefore, growth data were analyzed separately. Nevertheless, milk yield of heifers after first parturition were merged into a single data set and statistical analyses were performed on data collected for 204 lactations that followed first calving.

Experiment I

Animals. Sixty-four Holstein heifers weaned between 42-49 d of age were assigned to this experiment when they were between 50 and 155 d of age. They were assigned to one of four treatment groups (I-IV); 16 heifers were assigned to each group as they became available, but effort was made to balance the groups so that the range in heifer age and size within a group would not be too great. This was done so there would not be severe competition among heifers for feed. One heifer from treatment I died as a consequence of bloat at an older age (9 mo) and was not replaced. All heifers within a treatment group were housed and managed in a large pen (a total of 4 pens) with a feed bunk that provided greater than .7 m of linear space per heifer. The feeding area and a portion of each pen were covered by a metal roof (7.5 m wide). This provided some protection of the feed and heifers from rain and also provided shade for heifers from direct solar radiation, especially during the hotter months of the year.

Experimental design. The four groups of heifers were arranged in a 2 x 2 factorial design that consisted of two feeding programs formulated to support ADGs of .68 or .91 kg/d; one-half of the heifers within each of these groups was injected with bST or excipient from 220-340 d of age. Heifers remained on feeding programs until they were

ready to be bred, but bST injections were discontinued after 340 d of age. Breeding was initiated at the first estrus after they had reached 13 mo of age and when their body weight was 340 kg or greater.

Injections of bST. Beginning at 220 d and continuing through 340 d of age, two groups (II and IV) were injected subcutaneously over the shoulder area once daily with 15 mg of bST that contained 12.36 mg of the bST monomer. The bST was dissolved in sterile saline (15 mg/2 ml, pH=8.4) and refrigerated when not being used. Dissolved hormone was refrigerated and used within 5 to 7 d after it was prepared. Heifers in groups I and III received equivalent injections of the sterile saline excipient. Injections were in the morning before feeding or any other procedures. Hormone and diluent were provided by American Cyanamid Co., Princeton, NJ.

Feeding. The rations fed to heifers at the time they were assigned to the experiment consisted of corn silage and grain concentrate. All heifers started on corn silage feeding at about 80 d of age, even though they were not assigned to experiment until they were older. This was done to allow them to develop capability to digest a silage based ration before entering the growth study.

Required amounts of DM, energy (Mcal ME), CP and macro-minerals for groups of heifers was determined using the NRC (1989) computer program for heifers. The amount of feed delivered daily to individual groups was based upon the average weight of heifers in that group. In general, the NRC computer program requirements provided 60g CP/ME (Mcal) for heifers until they were about 210 kg BW and at greater BW this decreased to where the ratio was about 50g CP/Mcal ME or slightly greater. The

quantities of DM, CP, ME and ratio of g CP/ME (Mcal), offered for the two growth rates desired are in table 2-1.

Adjustments to feed offered for desired ADGs were made biweekly within several days after the weigh day. Composition of the corn silage and the two grain concentrates fed are in tables 2-2. Heifers were fed once daily between 0900 and 1000 h. Weighed amounts of corn silage were spread along the length of the feed bunk from a large feed wagon and it then was top-dressed with needed amounts of one or both grain concentrates. The proportions of the grain concentrate rations offered were changed in order to meet nutrient requirements for desired growth rates. Dry matter content of the silage was determined biweekly to adjust the amount fed. The silage and grain concentrates were mixed using a pitchfork to prevent, insofar as possible, uneven consumption of the grain and silage portions by heifers in the group. Uneaten feed, if any, was removed the following day but weights and composition of any refusals were not recorded. Because heifers were limit-fed the amounts of refusals were small. Water was available free-choice from water cups in each pen.

Animal measurements. All heifers were weighed and their heights at the withers measured when they were assigned to experiment and began receiving the experimental rations. Thereafter, at biweekly intervals on the same day of the week, all heifers were weighed and heights at withers measured before the morning feeding. All heifers were milked three times daily throughout lactation. Lactation records were utilized in subsequent statistical analyses if greater than 100-d long. Actual 305-d milk yields or

Table 2-1. Nutrient Requirements for Two Growth Rates for Growing Heifers[1]

Body Weight[2]	ADG[3]	DM (kg)	CP (g)	ME (Mcal)	CP/ME[4]
100	720	2.95	472	7.73	61.1
150		3.92	627	10.00	62.7
200		4.90	713	12.17	58.6
250		5.91	709	14.29	49.6
300		6.98	837	16.42	51.0
350		8.12	974	18.58	52.4
400		9.36	1123	20.81	54.0
450		10.73	1287	23.14	55.6
100	1000	3.52	563	9.21	61.1
150		4.63	741	11.81	62.7
200		5.76	867	14.30	60.6
250		6.93	846	16.76	50.5
300		8.17	980	19.22	51.0
350		9.50	1140	21.74	52.4
400		10.95	1319	24.35	54.0
450		12.56	1507	27.09	55.6

[1]NRC (1989). [2]Body Weight in kg.
[3]Calculated to give slightly greater nutrient intakes than of desired (720 and 1000g/d).
[4]CP/ME = ratio of crude protein intake (g) Mcal/ME.

longest record available and total milk yields were used in the analyses. Milk yield of 50 heifers that calved and met this criteria were used for the statistical analyses. All heifers were milked three times daily (0530, 1330, and 2130 h) in a Herringbone milking parlor

equipped with automatic animal identification and milk recording systems (Galaxy 2000). Milk weights were recorded daily and milk composition was from monthly DHIA sampling; analyses and records kept in the DRU. record keeping system.

Statistical analyses. Data for body weight and height at withers for the 50 Holstein heifers were analyzed by least squares analysis of variance using the general linear models procedures of SAS (SAS, 1991). Data analyses were performed in two ways. First, data collected from 90 to 365 d of age for heifers that calved and provided lactation records were analyzed and represented the overall growth period for the experiment. Thereafter, prepubertal growth periods were analyzed from 90 to 120, 90 to 180, 90 to 220, 120 to 220, and the peripubertal growth period from 220 to 365 d of age, the period of time when heifers were under influence of the bST or excipient injections.

Mathematical models used to evaluate overall growth rates from 90 to 365 d of age included treatment, season of birth of heifers, and the interaction of treatment*season of birth as sources of variation and either the initial weight or height as covariates in the analyses, as appropriate. The four treatments that were established for analyses were combinations of plane of nutrition and bST injections (I, control diet + no bST; II, control diet + 12.36 mg of bST/d; III, high energy diet + no bST; and IV, high energy diet + 12.36 mg bST/d). A second model was utilized to analyze data for the growth periods from 90 to 120, 90 to 180, 90 to 220, and 120 to 220 d of age. Because no bST injections had been utilized during this time period, these models only included plane of nutrition (diet), season of birth of heifers, and the interaction diet*season and either the initial weight or height as covariates, as appropriate. A third model was used to analyze data for

Table 2-2. Constituents and Composition of Corn Silage and the Two Grain Concentrates Fed to Growing Dairy Heifers, Dry Matter Basis[1].

Constituent	Corn Silage	Grain # 1 (%)	Grain # 2 (%)
Ground corn		70.70	36.80
Soybean meal		26.80	58.30
Dicalcium phosphate		1.69	3.07
Trace mineral salt		.72	1.84
Vitamin A (30,000 IU/g)		.03	0.03
Composition			
DM%	32.00	89.50	89.90
CP%	7.20	21.60	35.20
TDN%	63.00	82.10	78.70
ME (Mcal/kg)	2.38	3.10	2.98
Ca%	0.31	.47	.91
P%	0.22	.72	1.15

[1]From NRC (1989) and analyses at NEDHIA Forage Testing Laboratory, Ithaca, NY.

the growth period from 220 to 365 d of age, the period of time when bST was injected. This model included the effect of plane of nutrition (diet), bST, the interaction diet*bST, season of birth of heifers, and the two- and three-factor interactions of season with diet and bST and again either the initial weight or height included as covariates in the analyses, as appropriate.

Experiment II

Animals. One hundred and eighty-five Holstein heifers were assigned to experiment when they were between 50 and 120 d of age; of these 80 were assigned at 50 d of age. Two heifers died when they were 4-5 mo of age and they were not replaced.

There were two nutrition treatment groups for normal (control) and high ADGs, but numbers assigned to each group differed. Ninety-four heifers were assigned to control (.68 kg BW gain/d; NRC, 1989) and 89 to a high energy diet for rapid (.91 kg BW gain/d) ADGs which was determined by the amount and type of diet they were fed.

Heifers were raised in 14 groups that ranged from 10-16 heifers per group. They were assigned either when they were weaned or later, as they became available, such that heifers within a group did not vary greatly in size or age in an attempt to reduce unwanted competition at the feed bunk due to great differences in body size. All heifers were housed and managed together throughout the experiment. Heifers were provided with ample feedbunk space (>.7 m linear space/heifer), shade and had free access to water from water troughs or automatic water cups. The feeding areas were covered with either a metal roof or 80% shade cloth to provide some protection for feed and heifers from the rain and direct solar radiation, especially during hotter summer months.

Experimental design. The two growth rate groups were arranged, as indicated, so that there were 14 different groups of heifers. The 63 heifers that were assigned to the first four groups of 16 per group served as controls; they received no other treatment. Heifers in the remaining groups were arranged so that 76 received injections of recombinant bST (500 mg/14 d, subcutaneous) and the remaining 44 were not injected. Insofar as possible, numbers of heifers in a group that were injected or not injected were assigned so that, except for the first four groups assigned, there were multiple heifers in both the injected and not injected category for each of the groups, although not perfectly balanced. Because of numbers of heifers available and the desire to have a larger number

injected with bST for evaluation of their subsequent milk yields after first calving, some imbalance was unavoidable. After completion of injections (>365 d of age) all heifers were assigned to diets intended to support ADGs of .68 - .73 kg/d until the time they were bred. Breeding was initiated at the first estrus after heifers were 13 mo of age and they weighed 340 kg or greater.

Injections of bST. The bST was injected beginning 220 ± 3 d of age and continued for 10 injection cycles at 2-wk intervals. Therefore, heifers were under the effects of bST from about day 220 through day 360. The bST, prepared in an oil emulsion (500 mg/≅1.2 ml), and subsequently marketed under the name POSILAC for enhancement of lactation, was provided by Monsanto Co, St. Louis, MO. Injections were subcutaneous on alternating sides of the body just in front of or just in back of the scapula. Heifers were injected in the morning before they were fed.

Feeding. The feeding programs for the heifers from birth through the time they were assigned to experimental rations, formulated to support desired ADGs, differed depending upon the age they first were assigned to experiment. Heifers that were assigned at younger ages were fed a starter ration that consisted of grain concentrate containing about 10% ground alfalfa (table 2-3). The heifers assigned to the first six groups also had been on different colostrum feeding programs but were weaned by d 49 of age. These heifers and all others were on this diet until about 80 d of age and then they, along with other heifers assigned at older ages, were changed to a corn silage-alfalfa hay roughage mixture (6:1) and grain concentrate diet (table 2-4). Amounts of the roughage and concentrates fed depended upon the desired ADG of the heifers in the pen, as

established from NRC (1989) recommendations. By 100-120 d of age all heifers had completed the change to the corn silage-alfalfa, grain concentrate diet. The grain concentrate fed to heifers in the pens was top-dressed on the roughage mixture and not mixed further. The total amounts of feed delivered to individual pens were calculated to support the ADGs desired (NRC, 1989), and were based upon average weights of heifers in the pen at the previous weighing. Nutrient requirements for this weight were calculated using the NRC (1989) program considering DM, CP and TDN. Adjustments to feed offered, in order to obtain the desired ADGs, were made biweekly within several days after they had been weighed. The roughage mixture was spread along the linear feed bunk or added to a wooden feed bunk provided in the individual pens in the morning between 0900 and 1100 h daily. Because the animals were limit-fed there usually was no refused feed, but if there was it was removed and discarded, but weights and composition of the refused feed were not determined. Water was available free choice from water cups or troughs located in the pens.

Animal measurements. All heifers were weighed and heights at withers measured when they were assigned to experiment. Thereafter, biweekly on the same day of the week, they were weighed and heights measured. This was done before the morning feeding. Because a large number of animals were on trial at the same time it often was necessary to delay the feeding beyond 1100 h on days they were weighed; this usually represented less than a 2 h delay.

Table 2-3. Percentage of Constituents and Composition of Starter Ration Fed to Heifers up to 50 D of Age, Dry Matter Basis[1].

Constituent	IFN	Grain (%)
Ground corn	4-26-023	63.20
Soybean meal	5-04-612	22.60
Chopped alfalfa	1-00-063	10.30
Trace mineral salt	-	.56
Dynaphos	-	.56
Limestone	6-02-632	1.42
Aureomycin crumbles[2]	-	1.42
Rumensin-80	-	.031
Vitamin A (30,000 IU/g)	-	.031
Composition[3]		
DM		89.4
CP		20.1
TDN		78.5
Ca		1.08
P		.54
Mg		.19
K		1.06

[1]From NRC (1989).
[2]Central Soya, Fort Wayne, IN. Active ingredient Chlortetracycline, g/.454 kg. From NEDHIA Forage Laboratory analyses of three composites, Ithaca, NY

Table 2-4. Percentage Composition of Corn Silage-alfalfa Roughage (6:1), Fed to Growing Dairy Heifers, Dry Matter Basis[1].

Constituents	%
DM	41.10
TDN	65.30
CP	10.80
N.F.	49.40
ADF	34.70
Ca	0.63
P	0.25
K	0.19
Mg	0.19
S	0.12

[1]Calculated from book values (NRC, 1989) and analyses of individual ingredients at NEDHIA Forage Testing Laboratory, Ithaca, NY.

Milking and sampling. Heifers were milked as for experiment I and milk data collected similarly. Milk yields of 146 heifers that calved and completed at least a 100-d lactation period were used for the statistical analyses.

Statistical analyses. Data for weight and height of heifers were analyzed using the same divisions of the data by age and models as described for experiment I.

Results and Discussion

Growth data for weight and height for both experiment I and II were analyzed and reported, as previously, as individual experiments (Garcia Gavidia, 1995). However, because objectives of this study were to analyze milk yields of the first-calf Holstein heifers fed two planes of nutrition and injected or not with bST during the peripubertal growth period, only heifers that completed first lactation of not less than 100 d were included. Thus, only 50 heifers from experiment I and 146 from experiment II were considered. Because this number differed from the number previously evaluated (Garcia Gavidia, 1995), a new set of analyses were performed to establish the growth rates (ADGs) of those heifers that lactated and met the 100 d criteria. The same mathematical models and arrangement of data were used as previously described by Garcia Gavidia (1995).

Growth rates (ADGs) for the various growth periods are in table 2-5. During all prepubertal growth periods considered, heifers fed high energy diet (treatments II, IV, and VI) on average had greater ADGs than heifers fed control diet (treatments I, III, and V). These results agreed with previous reports that level of feeding of replacement heifers was the main source of variation on growth (Moran et al., 1989; Schillo et al., 1992). Although ADGs during the prepubertal growth periods fall within current recommendations of 0.6 to 0.8 kg/d for Holstein heifers (NRC, 1989), the group fed high energy diet did not grow at the expected ADG of 0.91 kg/d. Many factors can affect the rate of growth of animals at different ages; these include health, nutrition or a variety of

Table 2-5. Least Squares Means and SE for Rates of Gain (Kg/d) of Holstein Heifers During Various Prepubertal and Peripubertal Growth Periods[1].

	Treatments[2]					
Growth Periods	I	II	III	IV	V	VI
No. of animals	52	56	36	27	9	14
Prepubertal						
90 to 120 d	0.433 ± 0.09	0.538 ± 0.07	0.442 ± 0.10	0.580 ± 0.17	0.275 ± 0.20	0.194 ± 0.13
90 to 180 d	0.610 ± 0.03	0.709 ± 0.03	0.630 ± 0.04	0.694 ± 0.05	0.469 ± 0.06	0.568 ± 0.06
90 to 220 d	0.634 ± 0.03	0.746 ± 0.02	0.630 ± 0.03	0.699 ± 0.04	0.553 ± 0.05	0.751 ± 0.05
120 to 220 d	0.724 ± 0.03	0.821 ± 0.03	0.630 ± 0.03	0.707 ± 0.05	0.572 ± 0.06	0.843 ± 0.05
Peripubertal						
220 to 365 d	0.739 ± 0.02	0.815 ± 0.02	0.913 ± 0.02	1.031 ± 0.04	0.957 ± 0.04	1.010 ± 0.05
Overall						
90 to 365 d	0.719 ± 0.02	0.802 ± 0.01	0.817 ± 0.02	0.875 ± 0.03	0.752 ± 0.03	0.889 ± 0.03

[1] Heifers raised on two planes of nutrition and injected or not with either 12.6 or 35.7 mg bST/d.
[2] Treatments : I = Control diet - no bST; II = High energy diet - no bST; III = Control diet + 35.7 mg bST/d; IV = High energy diet + 35.7 mg bST/d; V = Control diet + 12.6 mg bST/d; VI = High energy diet + 12.6 mg bST/d.
Model: ADG = treatment, season, trt*season, adjusted by initial weight.

management practices. Management and nutrition likely are the factors in this study that were responsible for the lower growth rates during these peripubertal growth periods analyzed. Management and nutrition often are difficult to control because of changes in environmental conditions, feed quality, and incidence of health problems of short or long duration. Variations in quality of roughage may have occurred, especially because the length of the experiment was over several years and the roughage sources changed several times during this period. For example, new corn silage was made and fed at the DRU. each year. Also, bulk purchases of feed ingredients were necessary at frequent intervals. Grain concentrate rations used were prepared from the same bulk ingredients as used to prepare the herd rations and not from special purchases that were set aside solely for experiments I and II; this increased the frequency of ingredient purchases. Thus, the ratio of protein:energy intake could have differed from expected when feed requirements for the groups were calculated and, as a consequence, the ADGs were more variable and not as high as expected or desired.

Clearly, growth rates during prepubertal periods through 7 mo of age in both experiment I and II were not great enough to allow heifers to reach 50 % of mature body weight and show puberty at an earlier age. Nevertheless, during the peripubertal growth period from 220 to 365 d of age, the ADGs were either equal to and in some cases higher than projected by ration formulation and feed offered groups of heifers. Heifers on high energy diet and injected with either 35.7 (treatment IV) or 12.6 (treatment VI) mg bST/d, on average, had greater rates of gain (0.973 and 0.948 kg/d) than heifers also on high energy diet (treatment II: 0.815 kg/d) not injected with bST, and greater than heifers fed

control diet injected (treatment III:0.913, and V: 0.907 kg/d) or not injected (treatment I: 0.739 kg/d) with bST. It is important to note that the growth rates of the heifers that calved and lactated for at least 100 d were similar to the growth rates of the entire group of 183 heifers that originally were assigned to experiment and reported by Garcia Gavidia (1995). Thus, there was no evidence that heifers that lactated had grown either slower or faster during the peripubertal period than all bST injected heifers in the groups and that this was associated with their ability to conceive, calve, and lactate.

Growth of heifers is a relatively long-term process. Therefore, during the peripubertal growth periods amounts or proportions of organs and specific tissues may have differed within and across treatment groups. Increases in body mass occurs in three phases: early post-weaning period when growth is hypertrophic; during the prepubertal period when growth includes both hypertrophy and hyperplasia; and after puberty when hyperplasia is the dominant growth mechanism (DiMarco et al., 1987). The endocrine system largely controls body growth (Armstrong and Hansel, 1956), and ST is considered to be a key hormone associated with weight gains due to its anabolic, anti-INS, and lipolytic effects (Bass and Gluckman, 1990). Results of short-term and long-term studies using sheep, steers, cattle, and pigs demonstrated that ST enhanced feed utilization, increased nitrogen retention, and also resulted in improved ADGs (Brumby, 1959; Early et al., 1990; Enright, 1989; Grings et al., 1990; Machlin, 1972; Moseley et al., 1982; Sandles and Peel, 1987; Stelwagen et al., 1993; Turman and Andrews, 1955; Wagner and Veenhuizen, 1978; Wallace and Basset, 1966; Wynn et al., 1979). Therefore, it seems likely that injection of exogenous ST into heifers during the peripubertal growth period in

these two experiments altered the normal allometric pattern of tissue growth and this could have resulted in increased rates of protein accretion (muscle growth) and reduced rates of adipose tissue deposition (Machlin, 1972; Muir et al., 1983; Wagner and Veehuizen, 1978). Collectively, results of studies where ST has been given to growing animals indicate that ST is necessary for growth, and necessary for partitioning of nutrients to muscle or adipose tissue. Short-term actions of ST have been described as protein sparing (Pell and Bates, 1987) or for the preservation of protein in body tissues at the expense of fat mobilization to provide the energy needed when decreases in feed intake occur. Long-term actions of ST are to direct nutrients to target processes such as growth or lactation. Overall, stimulation of body growth induced by injections of bST probably is due to a combination of factors. These include changes in feed conversion efficiency, changes in metabolism of nutrients especially in the liver in order to generate more substrates for body use, or by changes in target-tissue metabolism related to the direct effects of ST in these specific tissues. Included in these changes are increases in synthesis of substrates which are available to growing tissues such as by gluconeogenesis to increase glucose availability to growing tissues. Also, ST acts to stimulate body growth via indirect effects through other hormones or growth factors such as INS or IGF-I. As a result of these activities of ST cells of various tissues undergo mitosis and protein synthesis and hypertrophy or cell expansion by protein accretion occurs. Thus, a unit of gain may be represented by different ratios of fat, protein and ash (Bonczek et al., 1988). From other experiments no definitive effects of ST on growth rates and body composition were apparent (Johnson et al., 1987; Murphy et al., 1991). ADGs during the overall

growth period (90-365 d of age) indicated that growth rates were increased by exogenous injection of 12.6 or 35.7 mg bST/d. Because no heifers were sacrificed and no body tissue composition results were obtained during the current study the exact effects of ST injections on body growth and growth of specific tissues can not be determined. However, there is no reason to expect that the feeding program used in the current study and use of bST differed greatly from the large number of studies where body growth and protein accretion were the response to bST.

Because treatment effects on BW during the peripubertal growth period (220 to 365 d) of age and during overall growth period (90-365 d of age) were significant ($P<0.001$), a second analysis was performed to partition the effect of treatment into single degree orthogonal contrasts. This analysis included the effects of bST, diet, and bST*diet on growth rate. Significant differences were detected for bST ($P<0.001$) and diet ($P < 0.05$) but the interaction was not significant.

Least squares means for growth rates due to plane of nutrition and bST are in table 2-6. Heifers fed high energy diet grew faster (0.932 vs. 0.876 kg/d) than those fed control diet. Similarly, heifers injected with 35.7 mg bST/d had higher ADGs (0.966 kg/d) compared to heifers injected with 12.6 mg bST/d (0.974 kg/d) and those not injected (0.789 kg/d). Again, these results indicated that dietary energy was the main source of variation during growth and that ST was important for partitioning of energy and nutrients during the peripubertal growth period.

Analyses of variance performed for data on actual or 305-d and total milk yields, adjusted for the ADG for each of the four prepubertal growth periods (90 to 120, 90 to

Table 2-6. Least Squares Means and SE for Rates of Gain (kg/d) of Holstein Heifers During Peripubertal Growth Period[1].

	No of animals	ADG [2]	
Plane of Nutrition			
Control diet	97	0.876 ± 0.02	
High energy diet	97	0.932 ± 0.02*	
Somatotropin (bST)			Contrast
0 mg/d (I)	108	0.789 ± 0.02	(I vs II +III)
12.6 mg/d (II)	23	0.947 ± 0.02	
35.7 mg/d (III)	63	0.966 ± 0.03	**

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2] ADG = average daily gain (kg/d).
* $P \leq 0.0505$
** $P<0.001$

180, 90 to 220, and 120 to 220-d of age) did not detect significant effects of ADG during any of these growth periods on measures of milk production of these first calf heifers (table 2-7). Similar lack of effect was observed when the overall growth period from 90-365-d of age was evaluated. However, ADGs calculated for the peripubertal growth period from 220 to 365-d of age, the time when bST was injected, were positively and significantly ($P < 0.05$) associated with both actual or 305-d and total milk yields (table 2-7). These analyses also showed that neither treatment, season of calving, or age at calving affected the actual or 305-d, or total milk yields. However, both actual or 305-d and total milk yields were significantly ($P < 0.001$) affected by year of calving. compared to heifers injected with 12.6 mg bST/d (0.974 kg/d) and those not injected (0.789 kg/d). Again, results indicated dietary energy was the main source of variation.

Least squares means and standard errors for milk yields of first calf heifers that had been treated during the peripubertal growth period are in table 2-8. Heifers in the six treatment groups had different growth rates during 220 to 365 d of age and also produced different amounts of milk. Heifers with higher growth rates tended to produce more milk. Actual or 305-d milk yields were 6641.3, 7024.8, 7006.9, 7076.1, 6706.3, and 6860.3 kg for treatments: I (control diet); II (high energy diet), III (control diet, injected with 35.7 mg bST/d), IV (high energy diet injected with 35.7 mg bST/d), V (control diet injected with 12.6 mg bST/d), and VI (high energy diet injected with 12.6 mg bST/d) Total milk yields for these six treatment groups were 7739.6, 8555.0, 8410.1, 8663.3, 8221.9, and

Table 2-7. Least Squares Analyses of Variance for Actual or 305-d and Total Milk Yields of First-Calf Holstein Heifers [1,2].

		Actual or 305-d Milk Yields			Total Milk Yield		
Source	df	SS	F	Pr > F	SS	F	Pr > F
Treatment [3]	5	9944414.3	0.28	0.9228	81474099.6	0.55	0.7402
Season of Calving	2	32696021.7	2.31	0.1017	26776718.0	0.45	0.6385
Year of Calving	3	180056954.7	8.50	0.0001	372422702.8	4.17	0.0069
Age at Calving	1	16480666.2	2.33	0.1284	49860418.7	1.67	0.1972
Growth rates (ADG)[4]	1	38422765.8	5.44	0.0208	108264890.9	3.64	0.0518
Error	181	1278525688.2			5387930536.5		

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2]Milk Yields (kg).
[3]Treatments : I = Control diet - no bST; II = High energy diet - no bST; III = Control diet + 35.7 mg bST/d; IV = High energy diet + 35.7 mg bST/d; V = Control diet + 12.6 mg bST/d; VI = High energy diet + 12.6 mg bST/d.
[4] ADG = average daily gain (kg/d).

Table 2-8. Least Squares Means and SE for Actual or 305-d and Total Milk Yields of First Calf Holstein Heifers [1].

Treatments[2]	No of Animals	ADG[3]	Actual or 305-d Milk Yields[4]	Total Milk Yield
I	52	0.758 ± 0.02	6841.3 ± 187.3	7939.6 ± 384.5
II	56	0.830 ± 0.03	7024.8 ± 170.1	8555.0 ± 349.1
III	36	0.928 ± 0.03	7006.9 ± 247.6	8410.1 ± 562.9
IV	27	1.030 ± 0.04	7076.1 ± 456.7	8663.3 ± 508.3
V	9	0.957 ± 0.04	6706.3 ± 387.6	8221.9 ± 937.4
VI	14	1.010 ± 0.03	6860.3 ± 274.2	7735.0 ± 795.6

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2]Treatments : I = Control diet - no bST; II = High energy diet - no bST; III = Control diet + 35.7 mg bST/d; IV = High energy diet + 35.7 mg bST/d; V = Control diet + 12.6 mg bST/d; VI = High energy diet + 12.6 mg bST/d. [3]ADG = average daily gain (kg/d). [4]Milk Yield (kg).

Orthogonal Contrasts:

I, II vs. III, IV, V, VI was not significant.
I, III, V vs. II, IV, VI was not significant.
I, IV, VI vs. II, III, V was not significant.
III, IV vs. V, VI was not significant.

7735.0 kg, respectively. Orthogonal contrasts of means were conducted for the following; I, II vs. III, IV, V, VI; I, II, V vs. II, IV, VI; I, IV, VI vs. II, III, V; and III, IV vs. V, VI. None of these contrasts was significant.

Potential for milk production is a direct function of the number of secretory cells and their synthetic activity during lactation, and of course, their ability to partition nutrients to the mammary glands during lactation (Forsyth, 1986; Knight and Wilde, 1987; McGrath, 1987; Tucker, 1981). Growth of mammary cells occurs in a pattern that is related to reproductive development during fetal life, puberty, pregnancy and lactation. Hormones importantly associated with mammary growth are ST, E_2 and PRL (Knight and Wilde, 1987; Tucker, 1981). Amount and type of mammary growth that occurs during different growth periods varied. During the pubertal period mammary gland growth is qualitatively and quantitatively less pronounced than that which occurs during pregnancy, but there is evidence that pubertal mammary development has a strong influence on future milk production of heifers after first calving (Foldager and Sejrsen, 1987; Johnson, 1988; Sejrsen, 1994; Sejrsen and Purup, 1997). Pubertal mammary growth (3 to 9 mo of age) is allometric because growth rates of the mammary glands are 1.8-3.5 times faster than those of the whole body. During this time the mammary fat pad and vascular system increase and the ductal epithelium of the parenchyma expands into the fat pad (VanAmburgh and Galton, 1994; Sinha and Tucker, 1969).

Plane of nutrition and concentrations of ST in plasma are two factors that have an effect on the contents of parenchymal tissue in the mammary gland during the prepubertal growth period. High ADGs before puberty negatively affect growth of mammary gland

parenchyma. Heifers fed for rapid growth rates during the peripubertal period had a 23% reduction in epithelial tissue weight and a 32 % reduction in DNA content. Several researchers concluded that this reduced epithelial tissue growth compromised the subsequent milk production of these first-calf heifers (Foldager and Sejrsen 1987, Niezen et al., 1996; Sejrsen et al., 1986, 1989). On the other hand, a growing number of researchers concluded that heifers could be raised at very rapid growth rates during the peripubertal period without compromising mammary epithelial growth or subsequent milk production (Capuco et al., 1995; Head et al., 1991; VandeHaar, 1998, Van Amburgh and Galton, 1994). There likely are several reasons for these contradictory findings. Perhaps most important is the fact that measures of milk production and epithelial growth during the peripubertal period have not been determined in the same animals, for obvious reasons. To measure epithelial tissue growth in the mammary gland during the growth phase the heifers would need to have been sacrificed and the measures made on tissues collected from them. Although measures by dissection and computed tomography procedures are quite good (Head et al; 1991), the subsequent milk production of these glands cannot be measured. The opposite problem exists where milk yields are measured. There is no real way to determine the growth of different cell types in the mammary glands of live animals during the peripubertal period to ensure that it had been depressed by the feeding program. Needed still are procedures to quantify mammary growth during the prepubertal growth period and the follow-up studies to measure milk production and to link the measures at these two physiological stages in the life of the dairy animal. Nonetheless, it is important to carry out research studies that link these separate

experiments together (growth rates and milk production) with defined feeding programs to establish practical programs to raise heifers for high milk production.

In this regard, actual or 305-d and total milk yields evaluated from these two growth studies were not affected by the level of feeding the heifers were on during the prepubertal growth period from 90 to 220-d of age. ADGs during this growth period fall within the actual range (0.6 to 0.8 kg/d) recommended (NRC, 1989), rates which do not result in negative effects on milk yield. However, actual or 305-d and total milk yields of heifers were greater for heifers with higher ADGs (0.973 kg/d) during the peripubertal growth period from 220 to 365-d of age. These results suggest that the deleterious effect of rapid growth on mammogenesis due to fat deposition did not occur and, thus the total milk yields were not decreased in the heifers that grew more rapidly. This agreed with previous findings (Head et al., 1991; Peri et al., 1993; Sejrsen and Foldager, 1992; VanAmburgh and Galton, 1994; Waldo et al., 1988). These results support the use of growth rates (ADG) of 1.0 kg/d recommended by many authors (Gardner et al., 1977; Kertz et al., 1987; Park et al., 1987; Wright and Russell, 1991).

Several explanations exist to account for results of the current study and other studies that rapid ADGs did not affect mammary growth and subsequent milk production. Two likely explanations are that the length of time during the prepubertal growth period when high ADG occurred may not have been long enough to cause the negative effect on mammogenesis, or the time period when the high ADGs occurred did not correspond with the exact time of allometric growth of the mammary gland. Neither of these reasons was effectively evaluated in the current study. There are two lines of evidence, one based

upon quantitative studies of mammary growth and the other on subsequent milk yields of heifers, that the allometric phase of mammary growth begins as early as 2 or 3 mo of age (Sejrsen and Purup, 1997; Sinha and Tucker, 1969). Clearly, the average ADGs of all heifers during the current study (table 2-5), as in other studies, was far less than 0.91 kg/d through 220 d, although it was for some individual heifers in each of the groups. Thus, the growth rates we observed may have been less than rates that inhibited ST secretion and mammary epithelial growth into the fat pad. If very rapid growth rates that begin at 2-3 mo of age are important for expression of the negative effect this would help to explain the variable results across studies. Very few of the heifer studies reported showed very high heifer growth rates (ADG) rates at this early age. It also could indicate that, as a practical consideration, detrimental effect of rapid growth would be less important than supposed because heifers usually are not raised to gain that much BW daily this early in life. However, if rapid growth rates that occur later in the allometric growth period can affect mammary epithelial outgrowth into the fat pad then it is important to consider possible causes for this effect so they can be remedied. Therefore, it is important to consider some of the physiological responses during this rapid growth period that might be involved in the effect on the mammary gland.

Hormonal changes that control mammary growth that may be mediated by the mammary fat pad may affect whether the appropriate signals are received by the fat pad so that signals from the fat pad to the epithelial tissue that stimulate or repress its growth are activated and there can be full development of mammary parenchyma (Sejrsen and Purup, 1997). Onset of puberty has been suggested as the time period when signals are

received by the mammary gland to return it to the isometric growth phase. In the current study, highest growth rates (ADGs) occurred during the peripubertal growth period. Doubtless, some of the heifers already had reached puberty and perhaps the mammary gland epithelial components were no longer susceptible to the effects of high energy intake and high rates of BW gain. If so, then it is even possible that the higher energy intake could lead to more mammary gland parenchymal growth, as suggested by Waldo et al. (1988). If there were a link between the rapid growth rate and the allometric growth phase and in the subsequent isometric growth phase with milk production after calving it would explain the lack of an inhibitory effect and the positive response of milk production observed in the current study. Another possibility is that the high rates of BW gain, due to feeding high energy diets, are not importantly associated with reduced growth of mammary epithelial components during the allometric growth period if the diet is well-balanced and contains enough CP (Sejrsen and Purup, 1997; VandeHaar, 1998). Whatever the true association between growth rates, hormones and mammary epithelial tissue growth, the actual and 305-d and total milk yields in the current study and in other studies tended to be equal or greater for heifers with the higher ADGs.

Least squares means for actual or 305-d and total milk yields also were calculated for the diet and bST groups as well (table 2-9). Heifers fed a high energy diet during the peripubertal growth period produced slightly but not significantly more actual or 305-d and total milk (6801.8 vs. 6916.3; 8133.0 vs. 8282.2 kg, respectively). Actual or 305-d and total milk yields of heifers injected with 35.7 mg bST/d, which had higher ADGs during the peripubertal growth period, were greater (6996.2 and 6841.3), compared to

those of heifers not injected (6839.7 and 8261.5) or injected with 12.6 mg bST/d (6841.3 and 7992) during the same growth period. These results suggest that injection of ST in some way either prevented appearance of any negative effect or enhanced mammogenesis in spite of a negative diet effect. Somatotropin likely has an important role in mammogenesis. Data from several studies have shown reduced serum concentrations of ST in prepubertal and pubertal dairy heifers fed high plane of nutrition (Capuco et al., 1995; Sejrsen et al., 1986; Sejrsen et al., 1982). Furthermore, exogenous administration of ST during the prepubertal and pubertal growth periods of heifers increased quantity of mammary parenchyma (Sandles et al., 1987; Sejrsen et al., 1986), overcame the negative effect of high level of feeding on milk yield of beef heifers (Hall et al., 1992), and increased milk yield of heifers treated with 20 mg bST/d prepartum (Stelwagen et al., 1992). Collectively, these data suggest that ST is an important, if not principal, controller of mammary tissue growth during the prepubertal growth period (Sejrsen, 1994).

Actions of ST on mammary gland are mediated by IGF-I. Serum concentrations of IGF-I are increased by exogenous administration of ST and thus, IGF-I could stimulate multiple cellular responses associated with synthesis of DNA, cellular protein and tissue growth (Clemmons and Underwood, 1991; Crooker et al., 1990; McShane et al., 1989; Purup et al., 1995). Mammary gland receptors for IGF-I have been demonstrated; these receptors change with the physiological state of the tissue. Thus, IGF-I receptors decline during the prepartum period, increase up to 75% with lactogenesis, which is associated with IGF-I binding with mammary microsomes, and

Table 2-9. Least Squares Means and SE for Actual or 305-d and Total Milk Yields of First-Calf Holstein Heifers[1].

		ADG[2]	Actual or 305-d Milk Yields[3]	Total Milk Yield
	No. of animals		(kg)	(kg)
Plane of Nutrition				
Control diet	97	0.876 ± 0.02	6801.8 ± 155.6	8133.0 ± 320.2
High energy diet	97	0.932 ± 0.02*	6916.3 ± 149.5	8282.2 ± 307.5
Somatotropin (bST)				
0 mg/d (I)[4]	108	0.789 ± 0.02	6839.7 ± 130.0	8261.5 ± 273.7
12.6 mg/d (II)	23	0.947 ± 0.04	6841.3 ± 328.1	7992.3 ± 675.1
35.7 mg/d (III)	63	0.966 ± 0.03	6996.2 ± 205.9	8478.9 ± 424.9

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2] ADG = average daily gain (kg/d).
[3]Means for actual or 305-d and total milk yields did not differ for plane of nutrition or for bST treatment and interaction was not significant (P>0.10). [4]Orthogonal Contrast I vs. II, II for ADG P<0.001
* P<0.0505

then decline again after parturition (Dehoff et al., 1988). Therefore, mammary gland responds to IGF-I acting directly on mammary epithelial cells to stimulate DNA synthesis and increase cell numbers.

Although the previous explanations support the role of ST as an important controller of mammogenesis, other researchers have reported contradictory results with no ability of ST to overcome the negative effect of high ADGs (Buskirk et al., 1996), no ability to increase mammary cell numbers (Binelli et al., 1995), and no ability to stimulate increased milk yield of first-calf heifers (Grings et al., 1990; Murphy et al., 1991; Stelwagen et al., 1992). Inconsistent and/or contradictory results of ST actions on mammary gland may be associated with the fact that circulating levels of IGF-I already are increased in heifers when they are fed high energy level. This argues against interpretation that ST is the main factor mediating the detrimental effect of feeding level on lactation potential (Breier et al., 1988). Additionally, mammary growth during pregnancy was not regulated at level of the type-1 IGF-I receptor (Winder et al., 1992), because there was increased binding of IGF-I to mammary microsomes (Dehoff et al., 1988). Instead, there is a strong possibility that the true relationship between ST, IGF-I and growth of mammary gland tissue may be modified, if not regulated, by IGF-I binding proteins and also by local production of IGF-I in the mammary gland (Sejrsen, 1994).

Overall, the results of the current study and earlier growth studies suggest that rates of gain from 0.7 to 1.4 kg/d during the peripubertal growth period did not negatively affect milk production of first-calf Holstein heifers during first lactation. Therefore, impairment of mammary gland due to high rate of gain during the peripubertal growth

period either did not occur or was overcome by exogenous administration of ST. Needed still are studies that consider the actual growth of the mammary parenchyma during the critical allometric growth period and the subsequent production by these same animals. This will require development of new quantitative analysis systems to evaluate mammary growth without sacrificing the animal and linking these measures to production responses, considering also the effects of specific components of the diet such as CP and energy, among others.

Because milk yield can be affected by many factors, a second series of data analyses was performed to evaluate their importance in the current study. This model included effect of bST, diet, season of calving, and year of calving as sources of variation and the effect of age at calving, calving weight, linear and quadratic effects of DIM, DO, and growth rates during the peripubertal growth period as a covariate. Least squares analyses of variance for actual or 305-d and total milk yields are reported in table 2-10. No significant effects were detected for bST, diet, age at calving or growth rates (ADG) on either actual or 305-d or total milk yields. Conversely, both actual or 305-d and total milk yields were significantly ($P<0.01$; $P<0.001$) affected by calving weight. The linear or linear and quadratic effects of day were detected for total milk yield ($P<0.001$) and for actual or 305-d milk yield ($P<0.001$), respectively. From these analyses a significant effect of DO was detected for both actual or 305-d and total milk yields ($P<0.01$; $P<0.05$, respectively).

Table 2-10. Least Squares Analyses of Variance for Actual or 305-d and Total Milk Yields of First-Calf Holstein Heifers [1].

		Actual or 305-d Milk Yields			Total Milk Yield[2]		
Source	df	SS III	F	Pr > F	SS	F	Pr > F
bST	2	2846061.9	0.29	0.7511	7486543.8	0.61	0.5420
Diet	1	1007960.4	0.20	0.6528	3921397.0	0.64	0.4234
Season of Calving	2	76407217.3	7.70	0.0006	42538798.2	3.49	0.0326
Year of Calving	3	41564693.5	2.79	0.0420	64576075.7	3.53	0.0160
Age at Calving	1	534517.3	0.11	0.7432	1180166.6	0.19	0.6603
Calving Weight	1	36244497.4	7.30	0.0076	67836990.0	11.14	0.0010
Days in Milk (Dim)[4]	1	137928268.9	27.79	0.0001	111381299.9	18.29	0.0001
Dim*Dim	1	83507479.1	16.82	0.0001	661194.3	0.11	0.7422
Days Open	1	39040748.1	7.87	0.0056	30861996.3	5.07	0.0256
Growth rates (ADG)[3]	1	904287.7	0.18	0.6700	2573480.2	0.42	.05165
Error	181	873555806.7			1071882317.9		

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2]Milk Yields (kg/d).
[3]ADG = average daily gain (kg/d).
[4]Sums of squares Type I for DIM and DIM*DIM, others Type III.

Results agreed with those reported previously that indicated that both prepubertal and postpubertal growth rates had significant effects on calving weights, but only postpubertal ADGs had a significant effect on milk yield (Hoffman and Funk, 1991). There was a positive relationship between body size at first calving and the first lactation milk yield. Heavier replacement heifers produced more milk during at first lactation (Heinrichs and Hargrove, 1987; Keown, 1986; Keown and Everett, 1986). The negative effect of accelerated growth rates appeared to be coupled with early breeding and calving at ages less than 22 mo (Hoffman et al., 1996; Lin et al., 1986). Physiological maturity of heifers is important at time of breeding to allow adequate mammary gland growth; thus, ADGs of about 0.8 kg/d followed with more rapid growth during pregnancy did not impair mammary tissue development (Daccarett et al., 1993; Harrison et al., 1983; Sejrsenet et al., 1982; USDA/DHIA, 1990). Therefore, it is important, at time of first parturition, that heifers weigh about 82% of mature body weight. Using this criteria for established calving weight the milk production can be optimized during first lactation. And importantly, these heavier heifers will be able to compete for nutrients at the feed bunk in the dairy herd situation and this will ensure a sufficient DMI. Heifers with smaller body size at calving usually have lower DMI and they will use some of the energy from the diet preferentially for growth instead of for milk production. Therefore, milk yield will be less even though persistency of lactation may be greater (VanAmburgh and Galton, 1994).

Actual or 305-d and total milk yields increased linearly with increase in DIM. As expected, these increases in total milk produced with advancing lactation followed the

trend usually expected for cows. Cows reach peak milk production at about 5 to 8 wk, thereafter persistency of lactation declines by about 3 to 6% per mo (usually the lower number for heifers) during the first half of lactation, so greatest portion of total lactation yield accumulates during this time period. During mid-lactation up to 240 d, persistency of milk production declines to about 8 to 10 % per mo and thus, increases in total milk yield occur at a lower rate. Finally, during late lactation through the end of the lactation loss in production is still high and may be influenced to a greater degree by pregnancy and nutrition, so the amount of milk produced does not represent an important percentage of the total produced to the end of the lactation. The relationship between DO and either actual or 305-d or total milk yields was linear.

Because of the significant effect of calving weight (CW), DMI and DO on actual or 305-d and total milk yields and because of the significant and positive effect of growth rates (ADG) during the peripubertal growth period from 220 to 365 d of age on these measures of milk yield, it was of interest to examine the relationship between the peripubertal ADGs and above variables (CW, DIM and DO). Three different models were used to analyze data for these three variables. Models included effect of bST, diet, season of calving, year of calving, age at calving, and ADG, as sources of variation and either CW, DO or DMI linear and quadratic, as applicable, as covariates. Least squares analyses of variance are in table 2-11. Analyses detected no significant effect of bST, diet, or season of calvings, on CW but highly significant effect on CW ($P<0.001$) was observed for year of calving, age at calving and growth rates during the peripubertal growth period.

Least squares means of CW due to rate of BW gain (ADGs) during the peripubertal period (plane of nutrition and bST injections) are in table 2-12. Heifers fed high energy diet had greater ADGs and, therefore, they were almost 8 kg heavier at calving than those heifers fed control diets (497.9 vs. 490.1 kg). Additionally, heifers injected with 35.7 mg bST/d with ADGs of 0.920 kg were 23.4 and 39.6 kg heavier at calving (530.3 vs. 506.9 and 490.7) than heifers injected with 12.6 mg/d of bST and heifers not injected, respectively. Growth rates of dairy heifers should be great enough to allow heifers to reach desired weights at desired ages and calve earlier (22-24 mo) with adequate body size. Results of these analyses showed that at calving the heifers were as heavy as recommended by others (Hoffman and Funk, 1992; Keown, 1986; NRC, 1989). Heifers from high producing herds have been reported to be heavier at calving (24 mo) than those of low producing herds (Heinrichs and Hargrove, 1987, 1991, 1994).

Analysis of variance (table 2-11) detected tendency for an effect of diet ($P<0.1$) and a significant effect for season of calving ($P<0.05$) on DMI. No effects were detected for year of calving or age at calving, but a highly significant effect of growth rate ($P<0.01$) during the peripubertal growth period was observed. Also found significant was the effect of DO ($P < 0.001$) on DIM. Least squares means for DIM for plane of nutrition and bST injections also are reported in table 2-12. Means for DIM relative to ADGs during the peripubertal growth period from 220 to 365-d of age showed that average DIM for heifers fed either control or high energy diets were similar (362.5 vs. 358.9). The least squares mean DIM for bST treatments were similar (363.4, 362.6 and 366.3 DIM) for heifers injected with 12.6, and 35.7 mg/d of bST, and not injected, respectively.

Table 2-11. Least Squares Analyses of Variance for Calving Weight, Days in Milk, and Days Open of First-Calf Holstein Heifers [1].

	Calving Weight			Days in Milk			Days Open		
Source	df	SS	F	df	SS	F	df	SS	F
bST	2	18345.5	1.25	2	10.9	0.02	2	4515.9	0.38
Diet	1	12072.6	1.64	1	865.5	3.12†	1	2333.4	0.39
Season of Calving	2	20617.9	1.40	2	1486.3	3.33*	2	37056.2	3.10*
Year of Calving	3	505524.5	22.92***	3	962.1	1.16	3	19594.3	1.09
Age at Calving	1	671517.1	91.32***	1	263.6	0.95	1	27770.7	4.64*
Growth rates (ADG)[2]	1	334062.2	45.43***	1	2003.3	7.22**	1	8861.7	1.48
Calving Weight (kg)		NA		1	580.8	2.09	1	16765.8	2.80†
Days Open		NA		1	9341.1	33.68***		NA	
Days in Milk (Dim)		NA			NA		1	219557.8	36.71***
Dim*Dim		NA			NA		1	253153.1	42.33***
Error	181	1330988.5		178	49366.3		177	1058633.8	

[1] Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg bST/d during the peripubertal growth period from 220 to 365-d of age.
[2]ADG = average daily gain (kg/d).
† $P < 0.1$
* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
NA = Not Applicable; not included in specific model

Table 2-12. Least Squares Means and SE for Calving Weight (Kg), Days in Milk, and Days Open of First-Calf Holstein Heifers[1].

	No of Animals	ADG (kg)	Calving Weight (kg)	Days in Milk	Days Open
		Means ± SE			
Plane of Nutrition					
Control diet	97	0.876 ± 0.02	490.4 ± 4.9	362.5 ± 8.0†	175.6 ± 9.6
High Energy Diet	97	0.932 ± 0.02*	497.9 ± 5.0	358.9 ± 7.7	168.1 ± 10.2
Somatotropin (bST)					
0 mg/d (I)[2]	108	0.789 ± 0.02	490.7 ± 4.4	366.3 ± 6.9	177.0 ± 8.7
12.6 mg/d (II)	23	0.947 ± 0.04	506.9 ± 10.8	363.4 ± 7.1	175.7 ± 21.7
35.7 mg/d (III)	63	0.966 ± 0.03	530.3 ± 6.7	362.6 ± 7.7	162.8 ± 13.6

[1]Heifers raised on two planes of nutrition and injected or not with either 12.6 mg bST/d or 35.7 mg/ bST/d during the peripubertal growth period from 220 to 365 d of age.

† $P<0.01$

* $P<0.0505$

[2]Orthogonal contrasts: I vs. II, III for calving weight ($P<0.01$); not significant for DIM and DO; but $P<0.001$ for ADG.

Finally, least squares analyses of variance for DO (table 2-11) showed a significant ($P<0.05$) effect for season of calving and age at calving, a tendency for a significant effect of calving weight ($P<0.1$), and highly significant ($P<0.001$) linear and quadratic effects of DIM. No significant effect of growth rate on DO was observed. However, least squares means (table 2-12) for DO due to rates of gain, indicated that heifers fed high energy diet during the peripubertal growth period had numerically but not statistically significant fewer DO (168.1 vs. 175.6) than heifers on control diet with lower ADGs. Moreover, heifers injected with 35.7 mg bST/d, with higher growth rates (0.920 kg/d) and thus heavier at calving, had fewer DO (162.8) compared to 175.7 and 177.0 d for heifers injected with 12.6 mg bST/d and those not injected. Therefore, results of this study suggest that bST injections during the peripubertal growth period had a positive effect on ADG, resulting in heavier heifers at time of parturition and decreased the DO after calving.

From the overall analyses of variance for actual or 305-d and total milk yields (table 2-7), it was shown that both measures of milk yield were influenced significantly by growth rates during the peripubertal period. In reduced least squares analyses model (table 2-10) when calving weight, DIM (linear and quadratic terms) and DO were included as covariates, these variables had significant effects on both measures of milk yield, but ADG was not associated with actual or 305-d milk yield but was with total milk yield; some of the independent variables were correlated with each other. Therefore, an extensive series of reduced least squares analyses of variance was performed to determine the relative importance of various independent variables (traits) on actual or 305-d and

total milk yields. Some of the results obtained from completing this series of analyses to calculate the standard partial regressions are shown in the path diagram in figure 2-1. Values in each path to milk yield or the other traits presented are standard partial regression coefficients and indicate the magnitude of effects on measures linked directly by arrows in the path. It is important to indicate that the known associations of traits are consistent in this path. Standard partial regression showed that the direct path from DIM to milk yield was the largest detected and is well-known to be a factor that affects total unadjusted lactation milk yields. Similarly, DO was large and agreed with expected association. As indicated, the relative size of the number was consistent with biological responses seen for cows during lactation. Importantly, ADG had little direct effect on milk yield but did have an effect on DIM. Other coefficients for effect of ADG on other traits in the path diagram were consistently small and for clarity of the figure these and others were not included. Overall, the standard partial regressions in the path diagram supports known associations among these traits. For example, DIM had the biggest effect on milk yield and the next largest effect was for BW; the only other direct path to milk yield. Effects of ADG and DO on milk yield were very small but ADG had a strong effect on DIM, as did DO. Moreover, ADG effects on calving weight also was fairly large, as might be expected for these data because heifers were raised on two planes of nutrition through one year of age.

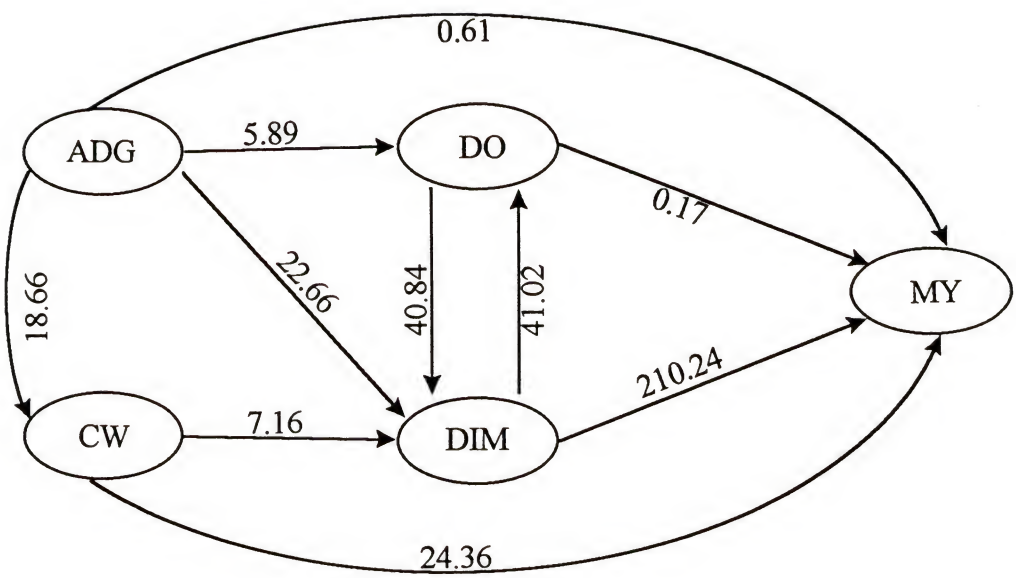

Figure 2-1. Standard Partial Regression Coefficients for Milk Yield as the Dependent Variable and ADG, CW, DIM and DO as the Independent Variables.

Conclusions

From this study it can be concluded that heifers raised at rapid growth rates between 90 to 365 d of age had greater body weight when they were fed the high plane of nutrition during the prepubertal growth period from 90 to 220 d, and during the peripubertal growth period from 220 to 365 d of age. Injections of bST during the peripubertal growth period increased growth rates irrespective of whether heifers were fed for normal or rapid growth rates, except when injected with the low dose of bST (12.6 mg bST/d). Furthermore, milk yields of heifers did not differ due to plane of nutrition or bST treatment during the peripubertal growth period and no interaction was detected. However, ADGs during the peripubertal growth period were significantly and positively associated with actual or 305-d and total milk yields.

CHAPTER 3
EFFECT OF NUTRITIONAL STATUS AND PREPARTUM AND POSTPARTUM BST TREATMENTS ON DRY MATTER INTAKE, ENERGY STATUS, BODY WEIGHT CHANGE AND MILK YIELD OF DAIRY COWS DURING EARLY LACTATION

Introduction

The lactating cow undergoes many physiological changes during the final weeks before calving and during the first few weeks postpartum. These time periods have been grouped together and termed the "transition" period. Changes that occur during the transition period, as cows move from the nonlactating to lactating state, impose enormous stress on them and may cause a reduction in dry matter intake (DMI), milk production and overall health status. Peak milk yield occurs from 5 to 8 wk postpartum but peak DMI may not be reached until 10 to 14 wk postpartum. Therefore, cows in early lactation may become deficient in energy (NE_L) intake and possibly in other nutrients. Reduced energy intake results in a negative energy status and typically there is increased mobilization of body fat reserves and labile protein by the dairy cow to supply the energy and amino acids needed for milk synthesis. As a result, most dairy cows lose body weight during early lactation. Additionally, during early lactation metabolic diseases may occur such as ketosis, fat cow syndrome, milk fever, acidoses, laminitis, and various reproduction associated diseases. The occurrence of one or more of these metabolic

diseases will have a negative effect on performance of lactating cows. It has been speculated that cows would produce more milk during lactation and have fewer incidences of metabolic diseases if DMI could be kept at a higher level during the prepartum period and/or during the early phase of the lactation.

Dry Matter and Energy Intake During the Transition Period

The transition period has been defined as the period from 3 wk prepartum to +2 wk postpartum (Nocek, 1996). It can be divided into two distinct phases: that from 5 to 7 d prepartum, which is characterized by a 30% reduction in DMI , and that from 0 to 21 d postpartum, a time period when the DMI should increase rapidly (Bertics et al., 1992). A reduction in DMI is initiated during the prepartum transition period, even though there is increased demand for nutrients to support conceptus growth and for the preparation of the initiation and increasing production during early lactation. Prepartum DMI is positively correlated with postpartum feed intake. Therefore, efforts to maximize feed intake should begin before parturition. Unfortunately, over-conditioned cows may be more susceptible to a prepartum decrease in feed intake (Grummer, 1995). Feed intake of Holstein cows during the final 27 d prepartum decreased from 1.8% of BW to 1.2 and 0.9% of BW, respectively, for normal cows and cows presenting one or more parturition associated or early postpartum disorders that required treatment. DMI for abnormal cows was significantly ($P < 0.10$) less than the DMI of normal cows from 3 d prepartum to 1 d postpartum (Zamet et al., 1979). These data suggested that reduced DMI prepartum increased cows susceptibility to disorders at calving and immediately after calving.

Although prepartum decreases in DMI seems to be unavoidable, the extent and duration of the decrease can vary (Emery, 1993; Grummer et al., 1995; Johnson and Otterby, 1981; Kunz et al., 1985; Vasquez-Anon et al., 1994). It was reported that DMI began to decline most dramatically beginning 1 wk prepartum (Bertics et al., 1992; Journet and Remond 1976; Lodge et al., 1975). Coppock et al. (1972) indicated that prepartum depression in DMI may be greater for cows consuming high grain diets than for those consuming high forages diets. Additionally, cows that were force-fed through a ruminal cannula also experienced feed intake reductions during the first 2 d after calving when orts were no longer placed into the rumen. This suggested that factors other than gut fill contributed to regulation of feed intake near the time of calving (Bertics et al., 1992). Identification of factors that influence prepartum feed intake is important. In the past, decreased intake often was attributed to reduced ruminal capacity associated with rapid fetal development during the last third of gestation (Forbes, 1968). In some instances, pregnant ruminants fed high-concentrate diets have exhibited decreased intake prepartum. However, the magnitude of prepartum intake changes may be less than expected based on the magnitude of decrease in ruminal capacity (Ketelaars and Tolkamp, 1992). Stanley et al. (1993) reported that at 22 d postpartum ruminal capacity was only 5% greater than at 61 d before calving, but postpartum DMI was 69% greater than DMI measured 61 d before calving.

Physiological factors, in addition to rumen capacity and distention *per se*, contributed to regulation of DMI during the transition period. Vasquez-Añon et al. (1994) reported that the decrease in DMI began 2 d before calving, at which time it was

only 40% of the DMI that was observed 3 d before feed intake depression, but plasma NEFA concentrations began to increase before DMI depression; decreases began 5 d before parturition. Stephenson et al. (1997) reported that cows treated with the ionophore monesin 50 d before expecting calving date had lower concentrations of glucose, NEFA, and β-hydroxybutyrate before calving but not after calving compared to cows not treated. Overall, metabolic adaptations of late-pregnant ruminants are characterized by increased hepatic gluconeogenesis but reduced glucose utilization in peripheral tissues, unchanged or reduced peripheral utilization of acetate, and moderately increased mobilization of NEFA from adipose tissue. These changes are associated with increased peripheral utilization of NEFA and the hepatic metabolite β-hydroxybutyrate (Bell, 1995). Thus, glucose sparing, which results in increased availability of glucose for conceptus metabolism, and the increased reliance of maternal tissues on NEFA and ketones for oxidative metabolism are among changes during the late transition period.

Psychogenic factors, which involve cows' behavioral responses to inhibitory or stimulatory factors in the feed or feeding environment, also have been described as modulators of DMI (Mertens, 1994). Palatability, social interactions, and learning behavior are integral components of psychogenic modulation of intake. Dado and Allen (1993) found that milk production of multiparous cows was related positively to meal size ($r = 0.75$) and length of eating bouts ($r = 0.75$), but was unrelated to meal number and eating rate, whereas for primiparous cows, milk production was related positively to meal number ($r = 0.55$) and eating rate ($r = 0.87$), but was unrelated to meal size. These results suggested that different mechanisms may be controlling individual meals and total

daily DMI among cows of different parity, rumen capacity or BW. Therefore, optimum intake during the transition period will occur only if feeding management accommodates normal feeding behavior of dairy cows (Grant and Albright, 1995).

Diet and Intake During the Transition Period

Rate of increase in DMI during early lactation may be the primary determinant of energy intake and balance (Chase, 1993). DMI increases by approximately 1.5 to 2.5 kg/wk during the first 3 wk of lactation (Bertics et al., 1992; Kertz et al., 1991; Nocek et al., 1983). Diet composition and nutrient content of the diet are factors that may influence prepartum DMI. Emery (1993) reported that DMI increased 30% when dietary NE_l was increased from 1.3 to 1.54 Mcal/kg of DM and CP was increased from 13 to 16% at 3 wk before parturition. However, during the final week preceding calving cows showed a DMI decrease, but they were still consuming 12.5 kg DM/d at calving. Proportion of grain concentrate in the diet also seems to affect DMI. Cows consuming 20% concentrate diet 28 d before calving had greater DMI from 28 to 11 d before calving than those consuming a 5% concentrate diet. Moreover, DMI of cows fed a greater proportion of high-moisture grain (47%) in a corn silage-alfalfa silage based diet increased from 28 to 13 d prepartum. However, the advantage not only began to decrease as parturition approached, but the decrease was more severe during the final week before calving and, consequently, there was no increase in DMI at calving (Coppock et al., 1972; Hernandez-Urdaneta et al., 1976; Johnson and Otterby, 1981; Kunz et al., 1985). Grummer et al. (1995) found that replacement heifers fed 6% concentrate during the 5 mo

period before calving had greater DMI during the final 10 d prepartum than cows fed 39% concentrate during the same period. Increasing the proportion of concentrates in the prepartum diet promoted development of rumen papillae and, thus, increased the capacity for VFA absorption, which, in turn, decreased ruminal VFA accumulation, reduction of ruminal pH, and susceptibility to acidosis. Because of these changes the DMI may increase (Dirksen et al., 1985).

Many research studies have evaluated the effect of energy density of prepartum diets on subsequent lactation performance. Researchers have reported that as energy density increased in the diet fed during the dry period, DMI also increased (Johnson and Combs, 1991; Johnson and Otterby, 1981; Nocek et al., 1986; Seymour and Polan, 1986). Enhancing intake of fermentable carbohydrates by increasing percentage of concentrate in the prepartum diet resulted in greater production of propionate in the rumen. As a result, hepatic production of glucose increased and this may have decreased glycogen depletion during the transition period (Grummer, 1993). Because propionate, and glucose to a lesser extent, are INS secretagogues (Harmon, 1992), if cows are responsive to INS near calving, then lipid mobilization from adipose tissue and glycogen depletion from the liver may be curtailed (Grummer, 1995).

It has been suggested that prepartum energy intake of cows should be restricted to reduce consumption of grain concentrate prepartum, in order to stimulate the type of metabolism that typically will occur immediately postpartum. Kunz et al. (1985) reported that DMI increased slightly faster, whereas energy deficiency and weight loss during the first week of lactation were reduced in feed-restricted cows. Additionally, feed-restricted

cows did not undergo prepartum DMI decrease. Instead, postpartum blood glucose increased, whereas NEFA and β-hydroxybutyrate decreased. Milk yield of feed-restricted cows was similar to that of cows fed forage *ad libitum* during the first 120 d of lactation. Cows that were fed diets that provided 67% of the NRC (1988) recommendations from 30 to 10 d before parturition had lower plasma concentrations of INS compared to those cows fed diets that provided 100 % of requirements (Lotan et al., 1988). This research suggested that reduction of INS in plasma may be advantageous because of reduced INS resistance and less lipogenesis. However, no effects on milk yield or on plasma glucose, NEFA or β-hydroxybutyrate were found. Nonetheless, a 20% restriction in feed intake starting 14 d postpartum increased the extent of negative energy status and caused transient increases in concentrations of NEFA, acetate, and β-hydroxybutyrate in plasma (Drackley et al., 1991, 1992).

Feeding supplemental fat has been recommended as a way to reduce fatty acid mobilization from adipose tissue during the transition period and during early lactation. Cows fed a high fat diet during the dry period showed decreased accumulation of triglycerides (TG) in the liver at d 1 postpartum, and this was accompanied by lowered concentrations of plasma NEFA (Grummer et al., 1996). However, in other studies plasma NEFA concentrations almost always increased when supplemental fat was fed (Chilliard, 1993; Grummer and Carroll, 1991). Moreover, feeding supplemental fat during early lactation neither reduced body weight loss nor influenced BCS during early lactation (Chilliard, 1993; Grummer et al., 1995; Hoffman et al., 1991; Jerred et al., 1990; Schingoethe and Casper, 1991). However, as indicated, feeding supplemental fat may

increase NEFA release from adipose tissue (Chilliard, 1993; Grummer and Carroll, 1991). This increased release may occur because of increased basal lipolysis, because of decreased reesterification of fatty acids, or for both reasons (Grummer, 1995). Nevertheless, feeding supplemental fat increased the speed of postpartum gain in BW once cows reached positive energy status (Harrison et al., 1995; Skaar et al., 1989). Seymour et al. (1994) concluded that feeding 5.5% dietary fat was optimal from d 1 to 49 of lactation, whereas 7% was best from 50 to 100 d of lactation. Lack of response to supplemental fat during early lactation seemed to be related to decreased DMI which eliminated the benefits of increased energy density of the diet when fat was added (Eastridge and Palmquist , 1988; Gagliostro and Chilliard, 1991; Grum et al., 1996; Ottou et al., 1995; Seymour et al., 1994). Moreover, Markus et al. (1996) reported no increase in DMI of dairy cows when they were fed diets containing either whole sunflower seed or tallow as the supplemental fat sources from d 16 to 16 wk of lactation. Lough et al. (1988) observed that DMI decreased with added fat when DMI was expressed in kg/d or as a percentage of BW; however, plasma concentrations of TG were increased, whereas concentrations of glucose, total protein and albumin were not affected.

Other strategies for feeding transition cows have been associated with manipulating protein intake of the periparturient cow. Van Saun et al. (1993) reported that improving amino acid status of the transition cow may affect performance, because listed NRC (1988) requirements may underestimate needs and thus, underfeeding protein may cause maternal reserves to be depleted. This could compromise lactation, health and reproduction of these cows. Improving amino acid status of periparturient cows may have

a positive influence on the endocrine physiology of cows and enhance lactation performance. Intravenous infusions of arginine 7 d prepartum increased concentrations of PRL, INS, and ST (Chew et al., 1983). Milk yield also increased about 10% during the first 23 wk postpartum. Komaragiri and Erdman, (1997) evaluated the effect of ruminally undegradable protein (RUP, 6 - 9%) on tissue mobilization and production parameters. They found that cows on both treatments mobilized a mean of 54 kg of body fat and 21 kg of body protein from 14 d prepartum to 114 d postpartum. Cows continued to mobilize 18 kg of body fat for an additional wk 12 postpartum, whereas body protein was unchanged. They concluded that increased RUP in the diet had no effect on the postpartum amounts of empty body protein, body fat or body energy. On the other hand, increasing UIP from 6 to 9% of DM between 2 wk prepartum and 16 wk postpartum increased plasma IGF-I and decreased ST during the postpartum period (Komaragiri et al., 1994). Additionally, Chew et al. (1984b) found that using urea to increase CP of a corn silage and alfalfa hay diet resulted in an increase in voluntary DMI of 0.1% of BW for cows consuming 100% of NRC recommendations for CP. Heifers fed diets containing blood meal to improve CP from 12.4 to 15.3% and UIP from 27 to 39% during the last 3 wk prepartum, maintained high body condition score (3.24; scale 1 to 5), produced more milk, and had fewer services per conception (Van Saun et al., 1993).

Treatment with bST from 15 to 44 wk postpartum increased DMI and 3.5% FCM yields. Milk yield increase occurred immediately after injections were started at wk 6, but increase in DMI was not evident until the end of wk 14 (Salfer et al., 1994). Thus, peak DMI lagged behind peak milk yield (McGuffey and Wilkinson, 1991). Chalupa and

Galligan (1989) found a 4-wk lag between the start of bST injections and the increase in DMI. Others (Lough et al., 1988; Lucy et al., 1993; Remond et al., 1991) reported that even though there was increased milk production and milk fat production no increase in DMI occurred in cows treated with bST from 44 to 105 d of lactation. Therefore, cows remained in negative energy status. Moreover, DMI of prepartum bST-treated cows tended to be 2.1 kg/d less than that of uninjected controls during the last week prepartum. DMI decrease was 1.9 kg greater for cows treated with 14 mg bST/d. However, during the first week postpartum DMI of bST-treated cows increased 3 kg/d but thereafter no differences among treatment groups occurred (Simmons et al., 1994). In contrast, Hartnell et al. (1991) showed that long-term treatment with bST increased DMI and NE consumption in a dose-dependent manner compared to controls. Increased voluntary DMI resulted in increased milk production and it supported the increased outflow of nutrients via milk (Jenny et al., 1992). However, feed intake, energy status, and corrected feed efficiency for milk production were not affected by bST injections (350 mg/14 d) from d 98 to the end of lactation (Gallo and Block, 1990a). In general, cows that previously had been injected with bST had higher postpartum DMI (Bauman et al., 1985; Hemken et al., 1991; Lean et al., 1991), lower plasma concentrations of NEFA and β-hydroxybutyrate, and higher plasma concentrations of glucose (Lean et al., 1991).

Differences in postpartum metabolism of cows may be attributed to differences in body composition resulting from bST treatment during the previous lactation, since these cows were in their second or greater consecutive lactation with bST treatment. Thus, bST-treated cows mobilized less lipid and produced more milk from feed rather than

from metabolites arising from mobilization of body tissue stores. Conversely, it has been reported that bST treatment increased plasma concentrations of NEFA and enhanced mammary gland uptake of TG from plasma (Lough et al., 1988). It can be concluded that administration of bST before parturition (-46 ± 6 d) increased protein reserves and stimulated milk protein synthesis for 1 wk postpartum but did not alter metabolism of subcutaneous fat. Thus, plasma concentrations of NEFA of treated and untreated cows were similar before and after parturition. Therefore, energy status was positive for all cows before calving. Energy status averaged 1.5 Mcal/d for cows injected with 5 mg bST/d but it was 1.1 and 3.8 Mcal/d for cows injected with 14 mg bST/d or for uninjected control cows (Simmons et al., 1994). After parturition, energy status decreased in all cows, but throughout lactation no differences in overall energy status occurred among groups. However, Lanna et al. (1995) found that cows injected with bST for 8 d during mid-lactation showed changes in net energy status from highly positive to slightly negative (7.7 to -1.1 Mcal/d). As a consequence, rates of lipogenesis and activities of acetyl CoA carboxylase and fatty acid synthetase enzymes were reduced in bST-injected cows. This indicated that when cows were in positive energy status there was reduced lipid synthesis in adipose tissue and this represented a major mechanism whereby bST altered nutrient partitioning to support greater milk synthesis.

Supplementation with bST significantly improved efficiency of conversion of feed into milk with no alteration of milk fat content and somatic cell count (SCC), but with lower milk protein yield (Remond et al., 1991). However, Lough et al. (1988) reported that injections of bST increased the percent of solids-not-fat (SNF) and tended to

decrease milk protein percentage with no change in lactose but increased milk fat yield and milk energy yield. Others (Gallo and Block, 1990a; Hartnell et al., 1991) have reported no changes in milk constituents of Holstein cows that were treated with different amounts of bST (350 and 250, 500 or 750 mg /14 d) from either 98 or 60 d postpartum throughout the lactation. Administration of bST early in the lactation (14 d to 60 d after calving) resulted in increased daily production of 3.5% FCM. Cows injected with 5 mg bST/d produced 1.2 kg/d more milk than cows injected with 14 mg bST/d, whereas the latter group of cows produced 1.3 kg/d more milk than uninjected control cows (Stanisiewski et al., 1992).

Body Condition Score and Transition Period

Repletion-depletion of body energy has been measured quantitatively as body condition score (BCS). BCS values using scales of 1-9 and 1-5 have been reported. The caloric value of live-weight change is influenced not only by gain or loss of body fat, but also by the replacement of body fat with water and by the change in the gut fill as well. In general, composition of tissue mobilized changes as lactation advances and the total amount of tissue mobilized increases. It has been reported that empty body water, protein, mineral, fat and fat percentage all decreased from prepartum to postpartum period, whereas gut fill of cows increased from prepartum to 5 mo postpartum (Martin and Ehle, 1986). At calving, the amount of body fat estimated by BCS has been used as an indicator of the energy available during early lactation to supply that needed to provide for the difference between dietary energy intake and the requirements for body

maintenance and milk production (Gearhart et al., 1990). Waltner et al. (1993) evaluated relationships between BCS and production variables of Holstein cows. They found that BCS decreased during early lactation and was restored during mid to late lactation, but the time to reach the nadir BCS differed depending upon the parity of the cows. Thus, nadir BCS was at 2 mo for 1st and 2nd parity cows and at 4 mo for 3rd or greater parity cows. The relationship between BCS at calving and FCM yield at 90 d postpartum was quadratic ($P < 0.0001$). Increasing BCS from 2 to 3 increased FCM by 322 kg; increasing BCS from 3 to 4 increased FCM by an additional 33 kg but increasing BCS an additional full unit (from 4 to 5) decreased FCM production by 223 kg. It also has been reported that BCS at calving did not influence milk production or reproductive performance (Pedron et al., 1993; Ruegg and Milton, 1995). One unit of BCS at calving was associated with 438 kg of mature equivalent production and with 422 kg of 305-d milk yield. Therefore, BCS has been used as a measure of lipid mobilization that has occurred during early lactation to support increased milk production.

Kertz et al. (1991) found that first lactation cows lost BW during the first 4 wk of lactation, but the energy deficit continued up to 13 wk. Older cows lost BW up to wk 7 and energy deficit continued until wk 15. Thus, BCS may be a better indicator of energy status in early lactation than BW, perhaps because it is not affected by gut fill as is BW. Staples et al. (1990) reported that dairy cows started gaining body weight after 8 wk of lactation. Data of Pedron et al. (1993) showed that recovery of BCS started at 10 wk postpartum for cows that had BCS of 3 or 3.5 at calving but was delayed until 12 wk postpartum for cows with BCS scores of 4. Moreover, BCS at calving had a significant

effect on BCS changes postpartum. The significant interaction of time and BCS at calving indicated that the effect of time on BCS was affected by BCS at calving; cows with higher BCS at calving (>3.5) appeared to lose BCS for a longer period than those with lower (3.25) BCS at calving (Ruegg and Milton, 1995).

Variable effects of bST treatment on BCS have been reported. Cows treated with bST postpartum from either 9 to 39 or 29 to 36 wk postpartum restored BCS more slowly than those not treated (Remond et al., 1991; Salfer et al., 1994; Tessmann et al., 1991). However, BCS was not affected by bST injections when compared with cows injected with growth hormone releasing factor (GHRF); bST-injected cows maintained a positive energy status (Dahl et al., 1991). However, Chilliard et al. (1991) reported that from 8 to 20 wk of lactation control and bST-treated cows lost 8.5 and 21 kg of body fat, respectively; from 20 to 39 wk, bST-treated cows gained more BW, water and estimated proteins but lost more BCS and estimated lipids than did the control cows. Moreover, cows injected with 14 mg bST/d from 14 to 60 d of lactation had lower BCS than cows injected with 5 mg bST/d or than controls not injected during the same lactational period (Stanisiewski et al., 1992). Prepartum treatment with either 5 or 14 mg bST/d did not affect BCS or thickness of subcutaneous fat and BW were similar (Simmons et al., 1994)

Many studies have indicated that over-conditioned cows are more likely to have poor appetites postpartum (Garnsworthy and Jones, 1987; Holter et al., 1990). However, if postpartum DMI is related to prepartum DMI, then it could be hypothesized that over-conditioned cows also would consume less feed prepartum (Grummer, 1995). Prepartum DMI of cows with highest BCS > 3.6 (1= thin, 5= obese) was approximately 1.5% of

BW, whereas DMI of cows with the lowest BCS (<3.6) was 2% of BW (Emery, 1993). The correlation coefficients between prepartum BCS at 17 d and DMI at 1 d prepartum or 21 d postpartum from three studies were -0.25 ($P<0.12$) and -0.45 ($P<0.006$). This data suggested a relationship between BCS and prepartum intake, but it did not confirm cause and effect (Grummer, 1995). However, it has been reported that increased energy density in prepartum diet also was associated with increased body condition, but postpartum DMI was variable (Boisclair et al., 1986; Holter et al., 1990; Johnson and Combs, 1991; Seymour and Polan, 1986). Others (Fronk et al., 1980; Garnsworthy and Topps, 1982) indicated that excess consumption of energy prepartum led to excessive BCS at parturition and that this resulted in poor appetites, lower milk yield and more health problems postpartum. Cows with BCS of 3, 3.5, and 4 at calving had maximal loss of 0.6, 0.8, and 1.05 points BCS. Thus, a loss of 165, 220, and 289 Mcal NE_l could be estimated for each BCS group, by assuming that a live weight change of 56 kg corresponded to 1-unit change of BCS and that this corresponded to -4.92 Mcal NE_L (Pedron et al., 1993). On the other hand, Holter et al. (1990) showed that under-conditioned cows at calving had less body weight loss postpartum with no effect on milk yield. Palmquist (1993) suggested that feed intake and efficiency of energy utilization may be improved and metabolic disorders minimized if cows calved in moderate body condition (2.5 on a scale of 1 to 5). This could be achieved by adjusting the energy of the prepartum diet to meet desired BCS of cows at calving. The postpartum performance of cows will be affected when they are thin (BCS < 3) because they fail to reach peak milk

production potential. For fat cows (BCS > 4) with high depression of DMI and excessive body weight loss, there are associated metabolic problems (Nocek, 1996).

The BCS at calving also has been related to blood parameters. Cows with a BCS of 4 at calving had higher plasma concentrations of NEFA at 30 d postpartum, whereas cows calving at BCS of 3 and 3.5 had plasma NEFA concentrations lower than 300 μeq/L, values that were considered to limit DMI (Pedron et al., 1993). Glucose concentration was not related to BCS at calving. On the other hand, cows with class 4 BCS had lower concentrations of INS than cows with class 3 BCS; concentrations of INS were lowest in high producing lactating cows.

Milk fat of cows calving at BCS of 3 had higher content of short-chain fatty acids and lower content of long-chain and unsaturated fatty acids; the BCS at calving was positively correlated with milk fat components derived from adipose tissue (long-chain and unsaturated fatty acids) and negatively related to fatty acids synthesized from acetate (Pedron et al., 1993). Conversely, the short-term increase in milk fat concentration of cows treated with bST was due to a change in energy status. Therefore, cows mobilized more body fat which increased blood concentrations of NEFA and, thus, increased uptake of NEFA by the mammary gland (Chalupa and Galligan, 1989).

Energy Status and Metabolism During the Transition Period

During early lactation high producing dairy cows typically are in negative energy balance because intake of dietary energy is less than the energy output for body maintenance and milk production. During pregnancy and lactation cows have a critical

period that lasts about 20 to 30 d which has the greatest influence on the entire lactation performance (Nocek, 1996). He defined this period to be within the time from 27 d before calving to 14 d postpartum. Physiological changes that occur during this critical period have a major impact on cows' health and how nutrients are utilized during the subsequent lactation. The transition between late pregnancy and early lactation presents an enormous metabolic challenge to high-yielding dairy cows and is marked by changes in endocrine status to accommodate parturition and lactogenesis. These changes influence tissue metabolism and nutrient utilization. Therefore, nutritional and other strategies to facilitate a satisfactory periparturient transition should be based upon a thorough understanding of the quality and quantity of nutrients required to support conceptus growth during late pregnancy and milk synthesis during early lactation (Bell, 1995). It is of crucial importance to consider the homeorhetic regulation of metabolic changes in non-uterine and non-mammary tissues such as the liver and adipose tissue.

The relatively high ratio of utilization of energy for milk synthesis to energy for maintenance requires that there be a cycle of energy storage during pregnancy, lipid mobilization and increased feed intake during lactation, and restoration of body fat during late lactation. During the periparturient period, adaptations take place in many endocrine organs and maternal tissues to supply milk precursors and energy-yielding compounds to the mammary gland. Thus, in the adipose tissue the rate of anabolic reactions slows during late pregnancy and is further reduced throughout the lactation. Adaptations of metabolism of adipose tissue throughout lactation are coordinated by the central nervous system through the endocrine organs and the sympathetic nervous system (McNamara,

1995). The intake of nutrients and demand for milk precursors have differential effects on the enzymes for lipid synthesis and release from the adipose tissue and vary with stage of lactation, nutrient intake, and genetic potential for milk production. Regulatory mechanisms include those for the mediation of homeorhetic control through altered tissue responses to homeostatic regulators such as INS and adrenergic agents (Bell, 1995).

Adaptations during late pregnancy include changes in maternal nutrient metabolism. There is increased rate of whole-body glucose production, mainly hepatic gluconeogenesis, but decreased glucose utilization in peripheral tissues; increased plasma concentrations of NEFA and ketones, which becomes more evident close to term and is sharply exaggerated if energy intake is voluntarily or involuntarily restricted (Petterson et al. 1994); and increased protein synthesis and reduced amino acid catabolism in the liver and a greater predisposition to muscle proteolysis (Bell, 1995).

Transition from pregnancy to lactation is well-marked by the sharply increased mobilization of NEFA from adipose tissue during and after parturition. Plasma concentrations of NEFA are correlated with rate of entry of NEFA into the bloodstream in lactating cows (Bauman et al., 1988; Pullen et al., 1989). Therefore, NEFA entry rate is representative of fat mobilization from adipose tissue and of body fat lost (Dunshea et al., 1988). Importantly, during the transition period about 50% of NEFA being released into the bloodstream are either oxidized or incorporated into milk triglycerides. However, some of the NEFA in blood are assumed to be lost indirectly via oxidation of ketones derived by hepatic synthesis from NEFA (Pethick et al., 1983). The corresponding balance between synthesis of TG and lipolysis is given by the net release

of NEFA from adipose tissue. In periparturient ruminants increased mobilization of NEFA may be the result of the suppression of *de novo* synthesis or uptake and, thereafter, by the esterification of fatty acids to form TG, by promotion of lipolysis, by reduction of the intracellular reesterification of fatty acids released by lipolysis, or by a combination of these metabolic changes (Bell, 1995). It has been reported that the intracellular reesterification of hydrolyzed fatty acids is minimal soon after parturition. Therefore, this likely represents the period of greatest net release of NEFA from adipose tissue during early lactation because soon after the parturition basal lipolysis is relatively unchanged in cows (McNamara and Hillers,1986). Increased adrenergic stimulation of lipolysis around parturition may be responsible for the very high plasma concentrations of NEFA (Grummer, 1993).

During the early postparturient period, high mammary demands for glucose to support lactose synthesis dominates the cows carbohydrate metabolism. Davis et al. (1979) reported that mammary blood flow, oxygen consumption, and uptake of glucose and acetate increased markedly between 2 d and .5 to 1 d prepartum, before further major increases occurred on d 1 postpartum. Moreover, uptake of glucose was greater and the magnitude and timing (.5 to 1 d prepartum) of this increase indicated the onset of milk secretion, because glucose is required for lactose synthesis and lactose is the most important osmotic solute in milk. During the postpartum period up to peak of lactation, great amounts of glucose arise from hepatic gluconeogenesis from propionate and dietary amino acids, glycerol released from adipose tissue lipolysis, lactate of dietary and endogenous origin, and finally from tissue protein mobilized. This latter source is crucial

during the first 5 wk following parturition (Bauman and Elliot 1983). On the other hand, during early lactation, when cows are in negative energy balance and circulating concentrations of NEFA are relatively high, mammary uptake of NEFA may account for a significant fraction of milk fat synthesis (Grummer, 1993; Miller et al., 1991). An additional source of fatty acids for mammary metabolism are the very-low-density lipoproteins (VLDL) that are derived from NEFA taken up by the liver and esterified to glycerol.

Mechanism of Metabolic Regulation During the Transition Period

Petterson et al. (1993, 1994) observed a diminished sensitivity of whole-body glucose utilization and a decreased responsiveness of lipolysis and NEFA mobilization to INS. The almost total suppression of adipose lipogenesis that occurred after onset of lactation in cows was associated with low concentrations of plasma INS and the almost complete loss of adipose responsiveness to INS, in terms of glucose or acetate utilization or fatty acid synthesis (McNamara and Hillers, 1986; Vernon and Taylor, 1988). Results of Faulkner and Pollock (1990), in early lactation ewes, showed no effect of INS on glucose metabolism, but there was decreased INS-induced suppression of plasma NEFA, glycerol, and α-amino nitrogen. Furthermore, Vernon et al. (1990) found that there was diminished ability of INS to stimulate hindlimb uptake of glucose in lactating ewes. These reports suggested that during late pregnancy fat mobilization was facilitated by the decreased ability of INS to promote lipogenesis and to oppose lipolysis. During early lactation there was a moderate degree of INS resistance in adipose tissue and

muscle; this promoted the mobilization of NEFA and amino acids and sparing of glucose (Bell, 1995).

During the periparturient period, lipolytic responses to adrenergic agents, including natural catecholamines, are altered. Change in lipolytic responsiveness to catecholamines did not occur until the periparturient period. The antilipolytic effect of the autocrine/paracrine factor, adenosine, on adipose tissue was enhanced during early lactation (Iliou and Demarne, 1987; Vernon et al., 1991). Therefore, the effect of periparturient events on lipolytic capacity of adipose tissue represented the balance between opposing actions of lipolytic (β-adrenergic agents) and antilipolytic (α-adrenergic agents, adenosine, other factors) effectors (Bell, 1995). Thus, ST, which was not an acute lipolytic agent, increased the responsiveness of adipose tissue to β-adrenergic agents. The ST may have acted by altering numbers or binding of β-receptors or the response to binding, or by altering the responsiveness of adipocytes to adenosine (Bauman and Vernon, 1993). Therefore, ST may have partially removed the antilipolytic action of adenosine and, in this way, increased lipolysis during early lactation (McNamara, 1995).

Homeorhetic hormones have been described as regulators of most of the metabolic adaptations that start weeks or days before parturition and are responsible for the endocrine induced changes that initiated and sustained lactogenesis. They also have additional roles in functions such as parturition and partitioning of nutrients for adipose tissue, liver and skeletal muscle. Estradiol-17β, which increases to peak concentrations 1 to 2 wk before calving, has been implicated in the reduced appetite of ruminants that

occurs during late pregnancy. It also may increase the susceptibility of mobilization of fatty acids from adipose tissue during late pregnancy no matter what the feed intake or energy balance of the animals (Forbes, 1986). Moreover, ST concentrations increase during late pregnancy, reach highest peak concentrations at calving and then decline postpartum to moderately elevated concentrations through early lactation. It exerts a powerful galactopoietic influence once lactation is established. ST also alters tissue response to INS and catecholamines. Another important effect of ST is to decrease rates of lipogenesis and activities of key lipogenic enzymes such as acetyl CoA carboxylase, which is the primary rate-limiting enzyme in fatty acid synthesis from acetate or glucose, by opposing tissue response to INS (Bauman and Vernon, 1993). The reduction in lipogenesis has been associated with reduction of INS receptors on adipocytes (Bauman and Elliot, 1983; Bauman and Vernon, 1993; Vernon and Taylor, 1988). However, ST does not alter INS binding to its receptor, but in some way at the post receptor level ST either inhibits the action of the second messenger or prohibits its synthesis (Bauman and Vernon, 1993). One mechanism by which ST may affect action of INS is through the alteration of INS uptake into the cell and its degradation by inhibiting the INS protease necessary for normal action of INS (McNamara, 1995). Therefore, ST is a primary homeorhetic regulator during the transition from pregnancy to lactation (Bauman and Vernon, 1993). Bernal Santos (1982) reported positive effects of ST on adipose lipolytic response to adrenergic agents *in vivo*. Chronic treatment of lactating dairy cows with ST produced an increased lipolytic responsiveness but did not affect sensitivity to epinephrine (Sechen et al., 1990). Furthermore, ST caused increased hepatic

gluconeogenesis *in vivo* and *in vitro*, possibly by decreasing ability of INS to inhibit gluconeogenesis.

As reviewed, ST is a homeorhetic controller that regulates the partitioning of nutrients. Thus it is involved in coordination of the metabolism of various organs and tissues. Changes in tissue metabolism involve both direct effects on some tissues and indirect effects mediated by ST-dependent somatomedins (IGF-I and IGF-II) for other tissues (Bauman, 1992). Plasma concentrations of IGF-I and II are partially under ST control. Nutritional status has been shown to play a major role in the regulation of the somatotropic-axis in both monogastric and ruminant species (Breier et al., 1986; Maes et al., 1983a,b; Phillips and Unterman, 1984).

Low circulating concentrations of IGF-I during early lactation were associated with low nutrient balances in cattle (Ronge et al, 1988; Spicer et al., 1990; Vicini et al., 1991). Response of IGF-I to bST treatment was greater during the dry period, when cows were in positive nutrient balance. Concentrations of IGF-II were not affected by either stage of lactation or restriction of nutrients; however, IGF-II concentrations tended to increase when cows were in positive nutrient balance during the dry period and when cows were fed diets containing an excess of protein and energy during lactation (McGuire et al., 1992a). Voluntary DMI increased in bST-supplemented cows. This increase in voluntary intake occurred a few weeks after initiation of bST supplementation and persisted during the time bST was supplemented. However, the magnitude of DMI increase depended upon the response in milk yield and the energy density of the diet (Chilliard, 1989).

IGF-I influenced proliferation and differentiation of granulosa cells *in vitro.* In rats, expression of IGF-I mRNA and the levels of IGF-I in the ovary varied during the estrous cycle; levels were increased between proestrus and estrus (Carlsson et al., 1989). Because of the negative correlation between energy balance and concentrations of circulating IGF-I in early lactation and the role of IGF-I in steroidogenesis, IGF-I has been implicated as a mediator of effects of energy balance on reproduction (McGuire et al., 1992b). Evidence of positive effects of the bST-induced increase of IGF-I on ovarian and luteal function and follicular growth is accumulating. Treatment with bST increased the number of 2-5 mm diameter antral follicles in mature heifers (Gong et al., 1991,1993b). It is likely that the relationship among bST, IGF-I and reproductive efficiency is sensitive to the nutrient and energy status of the animal. It has been suggested that energy level (glucose or other gluconeogenic metabolites that includes amino acids, at least for gluconeogenesis) may affect transcription of IGF-I mRNA, whereas protein (amino acids) may primarily control translation (McGuire et al., 1992a). High energy intake decreased plasma ST. The treatment with bST restored ST concentrations in plasma and increased IGF-I secretion from hepatic and non-hepatic tissues.

Increased BCS was directly associated with positive energy balance. An increase in energy balance was associated with increased concentrations of IGF-I in serum during early lactation. This increase in concentration of IGF-I was associated with increased secretion of progesterone (P_4) during diestrus of the first and second postpartum estrous cycle, but increased milk yield was associated with decreased serum IGF-I concentrations

and energy balance (Spicer et al.,1990). Thus, increasing DMI before parturition and during early lactation would be one way to increase energy balance and to maintain increased concentrations of IGF-I in serum during early lactation. One expected result would be that secretion of P_4 would increase and ovarian activity would increase. This may be useful to shorten the interval to first estrus postcalving and probably also would shorten the calving interval. Early postpartum treatment of dairy cows (d 14 after calving) with 5 mg/d of bST increased FCM production and perhaps reproductive performance, although this was not conclusive (Stanisiewski et al., 1992). Moreover, the bST increased DMI after a few weeks and also increased concentrations of IGF-I in serum.

The objectives of this research were to evaluate the effects of prepartum and postpartum nutritional status and treatment with low doses of bST during the transition period around calving and during early lactation on the energy status, DMI, BW, BCS, and milk yield of Holstein dairy cows.

Materials and Methods

Experimental Animals

Forty eight multiparous Holstein cows were utilized in this experiment. Cows were selected randomly from the dry herd of the Dairy Research Unit of the University of Florida approximately 4-5 wk before expected date of calving. Cows were housed and managed in a free-stall barn and trained to use electronic feed gates (Calan gates,

American Calan Inc., Northwood, NH). During the prepartum period all cows were fed the same diet (close up dry diet; Table 3-1) which was formulated for the average weight of the cows and NRC (1989) requirements for dry cows. Approximately 3 wk before expected calving all cows were assigned to one of two groups of 24 cows each. Treatments were: group 1, 24 cows not injected with bST and group 2, 24 cows injected with 5-8 mg bST/d (sustained release formulation, Posilac). Cows in both groups remained in the free-stall barn throughout the trial but had access to dirt loafing lot until they had calved.

After parturition, cows were assigned to a 2 x 2 x 2 factorial arrangement. Each of the two prepartum groups were divided in four treatment groups of 6 cows each. Treatments consisted of two diets, one contained no whole cottonseed (WCS) (0, table 3-2) and the other 15 % of ration DM as WCS (table 3-3). Within each diet group one-half of the cows were injected with 5-8 mg of bST/d equivalent of a slow-release long-acting bST preparation (Posilac; 70 - 98 mg bST/14 d injection cycle). The experimental design resulted in eight treatment groups as follows: I) cows not injected during prepartum period, fed a diet that did not contain WCS and not injected with bST after parturition; II) cows not injected during prepartum period, fed a diet with 15% WCS and not injected with bST after parturition; III) cows not injected with bST during the prepartum period, fed a diet without WCS and injected with 5-8 mg bST/d after parturition ; IV) cows not injected during the prepartum period, were fed a diet with 15% WCS and injected with 5-8 mg bST/d after parturition; V) cows injected with 5-8 mg bST/d during the prepartum period, fed a diet without WCS and not injected after parturition; VI) cows injected with

Table 3-1. Dry Matter Concentrations and Chemical Composition of Close-up Diet fed to Holstein Cows during Prepartum Period[1].

Ingredients (%)	%DM
Corn Silage	37.12
Hominy	22.24
Distillers Grains	7.44
Soybean Meal	7.44
Whole Cottonseeds (WCS)	7.36
Springer Minerals	6.69
Bermuda Hay	10.70
Trace Minerals	0.59
Dicalcium Phosphate	0.42
Composition	Percentage[2]
DM	56.54
CP	15.28
Sol P[3]	34.80
ADF	22.34
NDF	37.34
FAT	4.83
TDN	68.10
NE_L (Mcal/kg)	1.58

[1]From NEDHIA Forage Laboratory, Ithaca, NY, analyses of four composites.
[2]DM basis.
[3]Percentage of the CP.

5-8 mg bST/d during the prepartum period, fed a diet with 15% WCS and not injected after parturition; VII) cows injected with 5-8 mg bST/d during the prepartum period, fed a diet without WCS and injected with 5-8 mg bST/d after parturition; and VIII) cows

Table 3-2. Dry Matter Concentrations and Chemical Composition of TMRs Without Whole Cottonseed Fed to Holstein Cows During Early Lactation (0-65 d).

	I[1]	II[2]
Ingredients	%DM	%DM
Corn Silage	22.36	22.38
Alfalfa Hay	11.68	11.69
Cottonseed Hulls	5.56	5.57
Citrus Pulp	--------	9.94
Hominy	30.52	21.40
Distillers Grains	15.61	13.41
Soybean Meal	8.44	10.28
Mineral Mix	5.33	5.33
Limestone	0.50	--------
Chemical Composition	Percentage[3]	
DM	66.15	62.35
CP	16.03	17.18
SP	31.50	32.66
ADF	24.08	22.58
NDF	39.88	34.66
FAT	3.50	3.56
TDN	66.00	67.83
NE_L (Mcal/kg)	1.52	1.56

WCS=Whole Cottonseed.
[1]Fed from October to December.
[2]Fed from December to March.
[3]Dry matter basis.

Table 3-3. Dry Matter Concentrations and Chemical Composition of TMRs with Whole Cottonseeds fed to Holstein Cows During Early Lactation (0-65 d).

	I[1]	II[2]
Ingredients	%DM	%DM
Corn Silage	21.69	21.72
Alfalfa Hay	9.13	9.14
Cottonseed Hulls	5.56	5.57
Citrus Pulp	--------	9.94
Hominy	25.58	16.28
Distillers Grains	11.19	9.19
Soybean Meal	6.05	7.89
Whole Cottonseed (WCS)	14.93	14.95
Mineral Mix	5.33	5.33
Limestone	0.55	-------
Chemical Composition	Percentage[3]	
DM	68.98	63.77
CP	15.38	17.67
SP	30.00	33.43
ADF	28.38	25.33
NDF	41.28	37.16
FAT	6.15	5.83
TDN	67.25	68.29
NE_L (MCal/kg)	1.54	1.68

WCS=Whole Cottonseeds.
[1]Fed from October to December.
[2]Fed from December to March.
[3]Dry matter basis.

injected with 5-8 mg bST/d during the prepartum period, fed a diet with 15% WCS cottonseed and injected with 5-8 mg bST/d after parturition. Two cows died, one because of acute fatty liver after 1 wk of lactation and the other due to agalactia. No data were recorded for these two cows and they were not replaced because of availability of cows.

The experimental arrangement of treatments, diets and cow numbers in groups follows:

Treatment I	6 cows	0 bST prepartum	0 % WCS diet	0 bST postpartum.
Treatment II	6 cows	0 bST prepartum	15 % WCS diet	0 bST postpartum.
Treatment III	6 cows	0 bST prepartum	0 % WCS diet	+ bST postpartum.
Treatment IV	6 cows	0 bST prepartum	15 % WCS diet	+ bST postpartum.
Treatment V	6 cows	+ bST prepartum	0 % WCS diet	0 bST postpartum.
Treatment VI	6 cows	+ bST prepartum	15 % WCS diet	0 bST postpartum.
Treatment VII	6 cows	+ bST prepartum	0 % WCS diet	+ bST postpartum.
Treatment VIII	6 cows	+ bST prepartum	15 % WCS diet	+ bST postpartum.

Injections of bST

Beginning at 3 wk before expected date of calving, the cows scheduled to receive injections of bST were given the first injection. They received two injections of bST (Posilac; 70 - 98 mg bST/14 d injection cycle) at 14-d intervals (3 and 1 wk before calving). When cows calved they were injected that same day and thereafter at 14 d intervals; last injection was on d 56 of lactation. Thus, cows received a total of 7

injections of bST during the trial. Injections were subcutaneous on either side of the tail-head; they always were after any blood samples had been taken but were before morning milking and feeding.

Feeding program

Cows within treatment groups were fed their respective TMR once daily (1000 to 1100 h). Enough TMR was weighed into the feed bunk to provide for the nutrient requirements of lactating dairy cows according to body weight and average milk yield and also to allow for 5-10% weigh back or refusals of TMR. Treatment groups I, III, V, and VII were fed diet without WCS (table 3-2), whereas cows in treatment groups II, IV, VI, and VIII were fed a diet with 15% WCS (table 3-3). Cows had access to water free-choice; barns were equipped with fans which helped cool cows when environmental temperatures increased above 25°C.

Data Collection

Feed intake. Feed intake was recorded daily. Amount of feed was offered to individual cows in all treatment groups to provide at least 5-10 % weigh back or refusals daily. Refusals were collected and weighed before the next feeding and were used for feed intake adjustments and nutrient intake calculations. Formulated TMR diets were sampled twice weekly. Throughout the experiment feed samples were collected at the time cows were being fed. Samples of respective TMRs were collected and composited biweekly for nutrient content analyses. Feed analyses were at the NEDHIA Forage

Testing Laboratory, Ithaca, NY, and included : DM, CP, Soluble P (SolP), FAT, NDF, ADF, TDN, and NE_L.

Body weight and body condition score. Body weight was recorded at the time of selection of the cows, at calving, and thereafter at weekly intervals during the prepartum and postpartum periods through d 70 of lactation. Measures were in the morning before first milking. BCS (scale 1= thin to 5= fat; Wildman et al., 1982) was determined by two persons with score agreement of .25 pt. at the time of selection of cows and weekly, as for BW measures.

Milk samples. Milk samples (50 ml) for milk constituent analyses were taken weekly during three consecutive milkings (morning, afternoon and night). Samples were refrigerated, then sent to the DHIA approved milk testing laboratory in McDonough, GA. They were analyzed for contents of (protein) P, FAT, and Solids-not-Fat (SNF). The DM and ash contents of milk samples were determined by drying weighed quantities of milk sample for 24 h at 60 °C, reweighing them to determine DM content, ashing the remaining DM in a Muffle Furnace at 550 °C for 12 h, and reweighing the residual ash to calculate ash content. Milk yield was recorded at each daily milking from 3 d after parturition to d 70 postpartum.

Blood samples. Blood samples (two 8 ml samples) were collected from the tail vein into heparinized vacutainer tubes (10 ml) by puncturing the tail vein with a 20 gauge vacutainer needle. Samples were collected three times each week (Tuesday, Thursday and Saturday) during the prepartum and postpartum periods up to 70 d after calving. Blood samples were taken in the morning before the morning milking or feeding. After

collection they were immediately placed in an ice-bath and processed in the laboratory within 2 h. Hematocrit (% red blood cells) and plasma protein (g/dl) were measured for each sample before the samples were centrifuged. Small quantities of well-mixed whole blood were allowed to flow into duplicate capillary tubes, which were sealed and then placed in a Micro-capillary centrifuge and spun for 2 min at 2000 RPM. Hematocrit was read on the reader and the plasma fraction in the capillary tube was used to determine the plasma protein using a refractometer. Plasma was harvested from blood samples that had been centrifuged for 30 min at 3000 RPM in a refrigerated centrifuge. The plasma was stored at -20°C until assayed for plasma glucose, ST, INS, IGF-I, PRL, T_3, T_4 (before and after calving) and for P_4 for samples collected postpartum.

Net energy intake and energy status. Net energy intake (NEI) was calculated from the daily DMI (kg) times the net energy for lactation (NE_L) obtained from the feed analyses obtained for the specific TMR composites using the following formula:

NEI (Mcal/d) = DMI (kg/d) x NE_L of feed (Mcal/kg); (NRC, 1989)

Energy status during lactation period was calculated according to following equation (Staples et al., 1990): EB (Mcal/d) = NEI - NE_m - NE_{milk} + NE_{BWC},

where:

NEI = Net energy intake;

NE_m = Net energy for maintenance;

NE_{milk} = Net energy excreted in milk;

NE_{BWC} = Net energy for change in body weight.

E_m (Mcal) = $BW^{0.75}$ x (0.080 Mcal/kg BW + 10% activity); (NRC ,1989)

NE_{milk} (Mcal/kg) = FCM (kg) x (0.3512 + [0.0962 x % milk fat]); (NRC, 1989)

FCM = Milk yield (kg) x FCM(kg); (NRC, 1989)

FCM(kg) = 0.4 + 0.15 x % FAT

NE_{BWC} (Mcal) = BWC x 5.12 (for weight gain), or 4.92 (for weight loss); (NRC, 1989).

Statistical Analyses

Data from this experiment were analyzed as a sequential experiment in two sections. The first section of the experiment included data collected during the 3 wk prepartum and these data were analyzed as a complete randomized design (CRD). Data were analyzed by least squares analysis of variance procedures of SAS (1991). Analyses performed included those for energy status, DMI, change in BW and BCS. Data were analyzed for the overall prepartum period and also for specific days within the prepartum period (-14, -7, -3, -1 and 0). Models included the main effect of prepartum treatment (PRTRT), cow (PRTRT) and days to the cubic order polynomial for the overall prepartum period.

Data collected after parturition constituted the second section of experiment. These data were analyzed as a 2x2x2 factorial arrangement, because there were two prepartum treatments (groups I and II), two diets (0 and 15% WCS) and two bST treatments after calving (injected or not injected with 5-8 mg bST/d). Analyses performed included those for energy status, DMI, milk yield, 4% FCM yield, BW and BCS. Data were analyzed as an overall period from d 0 to 65 d postpartum. Specific analyses for first week after parturition also were performed for DMI, energy status, BW and BCS. Models included the main effect of prepartum treatment (PRTRT), diet,

postpartum treatment (PPTRT), the two-factor interactions of PRTRT *diet, PRTRT*PPTRT, and PPTRT*diet, and the three-factor interaction of PRTRT*diet*PPTRT. When appropriate for the analysis, the linear and quadratic effects of BW and/or BCS were included in analysis as a covariate(s); the quintic order polynomial regression for days also was included.

Results and Discussion

Because the objectives of this investigation were to evaluate changes during the transition period from 21 d before parturition and also through 65 d postpartum, the data initially were analyzed as two separate data sets, that prepartum and that from calving through d 65 postpartum. Data obtained during the period before calving included DMI, BCS and BW. Twenty four Holstein cows were assigned randomly to each of the two bST treatment groups before parturition. No differences were detected between bST treatment groups for DMI , BCS, BW or energy status during the prepartum period from d -21 to d 0 (calving day). Least squares means for measures evaluated during the prepartum period are in table 3-4. Average DMI 3 wk before calving was almost 15 kg/d for cows in both treatment groups. The treatment group of cows that had been injected with bST weighed slightly more (15 kg) than cows that were not injected. The BCS of bST- treated cows also was slightly but not significantly higher (3.63 vs. 3.43). As expected, all cows were in positive energy status throughout the prepartum period. Analyses of data for wk 2 before calving indicated that none of the measures differed from those seen at wk -3 prepartum; cows in the two groups had similar DMI and BW.

Table 3-4. Least Squares Means and SE for DMI, BW, BCS and Energy Status of Holstein Cows During the Transition Period, (-21 d to Calving)[1].

	Overall (-21to 0)	SE	Wk-3	SE	Wk-2	SE	Wk-1	SE
DMI (kg)								
bST[1]	13.92	± 0.14	14.92	± 0.21	14.57	± 0.20	12.29	± 0.25
No bST	13.84	± 0.14	14.44	± 0.21	14.66	± 0.20	12.42	± 0.25
BW (kg)								
bST	715.61	± 0.55	706.85	±0.71	718.82	± 0.30	721.16	± 0.93
No bST	700.48	± 0.55	689.49	± 0.71	702.08	± 0.30	709.85	± 0.93
BCS								
bST	3.71	± 0.01	3.63	± 0.01	3.73	± 0.01	3.78	± 0.01
No bST	3.56	± 0.01	3.43	± 0.01	3.57	± 0.01	3.68	± 0.01
Energy Status ($Mcal \cdot d^{-1}$)								
bST	9.58	± 0.23	11.33	± 0.33	10.57	± 0.32	6.85	± 0.40
No bST	9.70	± 0.23	10.81	± 0.33	10.99	± 0.32	7.30	± 0.40

[1]5 mg bST/d starting 3 wk before parturition.
DMI=dry matter intake, BW=body weight, BCS=body condition score.

Table 3-4.-- Continued.

	d-7	SE	d-5	SE	d-3	SE	d-1	SE	d-0	SE
DMI (kg)										
bST[1]	14.67	± 0.25	13.04	± 0.30	11.62	± 0.46	9.44	± 0.65	9.52	± 0.65
No bST	13.75	± 0.25	13.15	± 0.30	12.48	± 0.46	9.11	± 0.64	9.32	± 0.64
BW (kg)										
bST	724.03	± 0.93	724.11	± 1.20	721.62	± 2.41	711.91	± 2.66	663.61	± 2.66
No bST	708.12	± 0.93	710.71	± 1.20	710.47	± 2.40	707.86	± 2.64	666.59	± 2.64
BCS										
bST	3.76	± 0.01	3.77	± 0.01	3.79	± 0.02	3.78	± 0.02	3.47	± 0.02
No bST	3.63	± 0.01	3.67	± 0.01	3.69	± 0.02	3.72	± 0.02	3.44	± 0.02
Energy Status ($Mcal \cdot d^{-1}$)										
bST	10.64	± 0.40	8.06	± 0.50	5.81	± 0.74	2.40	± 1.03	3.21	± 1.03
No bST	9.43	± 0.40	8.42	± 0.49	7.42	± 0.74	2.04	± 1.02	2.94	± 1.02

One week before calving, although cows still had similar BW, there was about 16 % decrease in the average DMI observed for both bST-treated and not treated cows; DMI was about 12.4 kg/d for each group compared to 14.8 kg/d at wk -3. Although DMI decreased, the BCS was slightly higher (3.78 and 3.68) for bST-injected than for the group of cows not injected. On the other hand, energy status during the week before calving also began to decrease, but still was positive (6.85 and 7.30 Mcal/d) for both injected and not injected cows, respectively. Because of the decrease in the average DMI during the week before calving and because feed intake of the cows is an important determinant of fat mobilization, it was of interest to identify critical days when the decrease in DMI began. Therefore, analyses for specific days (-7, -5, -3, -1 and 0) also were performed. Results (table 3-4) indicated that bST-treated cows had a 20.7% decrease in DMI from d -7 to d -3, compared to a 9.2% decrease in DMI for cows not treated with bST. On d -1, as on d 0, DMI for both groups of cows was about 65 % of that seen on d -7. These results agreed with those previously reported that there was a dramatic decrease during the week preceding calving (Journet and Remond, 1976; Lodge et al., 1975), and with results that showed a 38 to 40% decrease during the last week before calving (Bertics et al., 1992; Vasquez-Añon et al., 1994), but differed from results presented by Simmons et al. (1994). They reported greater decrease in DMI for cows injected with bST (5 and 14 mg/d) before calving. In part, this difference may have resulted from higher dose of bST injected in one group in their study. Although DMI decreased during wk-1, this was not associated with high incidence of metabolic diseases during early lactation, which differed from report of Zamet et al. (1979). During the

present experiment one cow presented displacement of abomasum and another cow had clinical ketosis. Interestingly, both of these cows were from the group of cows that had not been treated.

Causes of decreased DMI during the late prepartum period have been explained by the series of metabolic adaptations that occur during late pregnancy. Increased hepatic gluconeogenesis, reduced glucose utilization by peripheral tissues, reduced utilization of acetate and slightly increased mobilization of NEFA and subsequent peripheral utilization of NEFA and β- hydroxybutyrate from the liver result in an increased availability of glucose for conceptus metabolism. However, the maternal tissues rely on the metabolism of NEFA and ketone bodies (Bell, 1995). These metabolic adaptations seem to be regulated by mechanisms which include mediation of homeorhetic control by altering tissue responses to homeostatic regulators such as INS and adrenergic agents. Thus, the increased mobilization of NEFA during the transition period seems to be the result of a decrease in *de novo* synthesis of TG, by the uptake and subsequent reesterification of fatty acids from TG; by an increased lipolysis; or because reduction in the intracellular re-esterifcation of fatty acids released by lipolysis. Most likely it resulted from a combination of these changes (Bell, 1995). Nonetheless, these changes are associated with decreased ability of INS to promote lipogenesis and to oppose lipolysis (Vernon et al., 1990). Moreover, homeorhetic hormones have been implicated as regulators of metabolic adaptations during late pregnancy. Thus, estradiol-17 β (E_2,) which shows high plasma concentrations during late gestation, has been implicated in the reduced DMI of ruminants before calving and also to be associated with the greater susceptibility of

NEFA mobilization from adipose tissue during this time period (Forbes, 1986). Additionally, concentrations of ST begin to increase during late pregnancy. Actions of ST resulted in decreased rates of lipogenesis by reducing INS receptors on adipocytes, or by inhibiting the action of the second messenger, or it could inhibit the INS protease required for normal actions of INS. For these reasons, ST is considered the major regulator of metabolic adaptations during the transition from late pregnancy to lactation (McNamara, 1995) and, indeed, throughout the lactation period.

After parturition the data were analyzed excluding the prepartum period to focus on the effects of nutritional and bST treatment on the initiation of the lactation and the maintenance of the lactation. Least squares analyses of variance for DMI, energy status, body weight change (BWC), BCS, milk yield, 4 % FCM yield and milk constituents were performed separately. Results of analyses for each dependent variable are presented and discussed together.

Dry Matter Intake, Energy Status, and Changes in Body Weight and Body Condition

After parturition each of the two prepartum treatment groups of cows were divided into two groups that were fed different diets, each diet group contained 12 cows, and within the diet groups half of the cows received injections of 5 mg bST/d. Analyses performed for the dependent variables after d 3 postpartum included the main effects of prepartum treatment (PRTRT), diet, postpartum treatment (PPTRT), the two- and three-factor interactions of PRTRT, diet and PPTRT, cow(PRTRT*DIET*PPTRT) and included days to the quintic order polynomial regression when analyzing the overall

postpartum period (0 to 65 d). As was done for wk -1 prepartum, a series of analyses was performed for specific days (1, 3, 7, 14 and 21) during the first 3 wk after calving.

Least squares analyses of variance for DMI and energy status for the overall lactation period from 0 to 65 d are in table 3-5. No effects due to PRETRT, diet, or PPTRT were detected for DMI and energy status. No significant effects were detected for the two-factor interactions of PRETRT*diet and PPTRT*diet, nor for the three-factor interaction of PRETRT*diet*PPTRT. However, the two-factor interaction of PRETRT*PPTRT was significant (P<0.0281) for DMI and approached significance for energy status (P<0.1038). A significant effect to the quintic order polynomial for day was detected for both DMI and energy status.

Least squares analyses of variance for BW and BCS during the overall postpartum period (0 to 65 d) are in table 3-6. No significant effect of PRETRT or diet were detected; however the interaction PRETRT*diet was significant for BW (P<0.0759) but not for BCS. In addition, the main effect of PPTRT was significant for BW (P<0.1004) but not for BCS, whereas the interaction PRETRT*PPTRT was significant for both BW (P<0.0859) and BCS (P<0.0371), and the three-factor interaction of PRETRT*diet*PPTRT was significant (P<0.0445) for BW. There were significant linear (P< 0.0078) and quadratic (P < 0.0012) effects of BCS on BW and significant linear (P<0.0932) and quadratic (P<0.0001) effects of BW on BCS detected, as well (P<0.0932, P<0.0001). Finally, the cubic polynomial for days was significant for BW but not for BCS.

Table 3-5. Least Squares Analyses of Variance for Dry Matter Intake and Energy Status of Holstein Cows During Early Lactation (0-65 d).

	Dry Matter Intake				Energy Status		
Source	df	MS	F	P>F	MS	F	P>F
Pretrt[1]	1	366.14	0.47	0.4965	1138.30	0.32	0.5778
Diet	1	1757.13	2.26	0.1408	1227.76	0.34	0.5633
Pretrt * Diet	1	39.20	0.05	0.8235	5670.56	1.57	0.2179
PPtrt[2]	1	1915.35	2.47	0.1246	37.88	0.01	0.9190
Pretrt * PPtrt	1	4049.34	5.21	0.0281	9464.80	2.62	0.1038
Diet * PPtrt	1	1015.99	1.31	0.2599	196.89	0.05	0.8167
Pretrt * diet * PPtrt	1	1166.01	1.50	0.2280	6909.94	1.91	0.1747
Cow (Pretrt * Diet * PPtrt)	38	776.66	49.86	0.0001	3612.17	9.83	0.0001
Days (D)[3]	1	23211.01	1490.18	0.0001	215812.75	587.40	0.0001
D * D	1	3844.65	246.83	0.0001	13707.20	37.31	0.0001
D * D * D	1	1239.57	79.58	0.0005	1702.38	4.63	0.0314
D * D * D * D	1	188.67	12.11	0.0006	2102.81	5.72	0.0168
D * D * D * D * D	1	148.08	9.51	0.0021	3327.53	9.06	0.0026
Error	2940	15.57			367.40		

[1]Prepartum Treatment.
[2]Postpartum Treatment.
[3]Type I Sums of Squares for all D terms; others are Type III.

Table 3-6. Least Squares Analyses of Variance for Body Weight and Body Condition Score of Holsteins Cows During Early Lactation (0-65 d).

Source		Body Weight			BCS		
	df	MS	F	P>F	MS	F	P>F
Pretrt[1]	1	23378.37	0.26	0.6109	0.180	0.07	0.7999
Diet	1	1028.53	0.01	0.9149	0.090	0.03	0.8583
Pretrt * Diet	1	295682.89	3.33	0.0759	6.750	2.46	0.1248
PPtrt[2]	1	251779.94	2.84	0.1004	2.490	0.91	0.3463
Pretrt * PPtrt	1	276029.02	3.11	0.0859	12.790	4.67	0.0371
Diet * PPtrt	1	90252.69	1.02	0.3198	1.810	0.66	0.4220
Pretrt * Diet * PPtrt	1	383655.35	4.32	0.0445	7.220	2.64	0.1127
Cow (Pretrt * Diet * PPtrt)	38	88798.75	563.67	0.0001	2.740	214.72	0.0001
BCS OR BW[3]	1	11668779.64	7407.04	0.0001	74.360	58.28	0.0001
BCS * BCS or BW * BW[4]	1	15437.28	97.99	0.0001	0.272	21.31	0.0001
Days (D)[5]	1	76028.07	482.61	0.0001	0.040	2.82	0.0930
D * D	1	37364.04	237.18	0.0001	0.098	6.81	0.0091
D * D * D	1	2972.90	18.87	0.0001	0.002	0.23	0.6298
Error	2942	157.54			0.013		

[1]Prepartum treatment. [2]Postpartum treatment. [3]Linear effect of either BW or BCS as appropriate in respective analyses. [4]Quadratic effect of either BCS or body weight as appropriate in respective analyses.
[5]Type I Sums of Squares for all D terms; others are Type III.

Least squares means and errors for the effect of the two-factor interaction of PRETRT*PPTRT on DMI, energy status, BW and BCS are in table 3-7. Also included in table 3-7 are least squares means for DMI as a percent BW, NE_L intake, NE_m, body weight change (BWC), NE_{BWC}, and NE_{MILK}. Results showed that cows injected with 5 mg bST/d during the prepartum period and also during the postpartum period had greater DMI than cows that were injected only during prepartum period (23.69 vs.19.08 kg/d); means also were greater than for cows not injected during the prepartum period whether injected or not after parturition (23.69 vs. 19.97 and 20.21 kg/d). However, DMI of cows not injected during the prepartum period was slightly higher than DMI for cows not injected during the prepartum period but injected after parturition (20.21 vs. 19.97 kg/d), and for cows injected prepartum but not injected during the postpartum period. Contrasts of these means indicated that the interaction resulted because of bST injections after parturition ($P<0.1$). Similar to results for DMI, for the DMI expressed as a percentage of BW, the cows treated with bST both prepartum and postpartum consumed more DM than cows injected prepartum but not injected postpartum, and more than cows not injected prepartum but either not injected or injected postpartum, respectively (3.7 vs. 3.2, 3.5 or 3.3% of BW).

Regression analyses were performed for DMI to describe trends in consumption throughout the postpartum period for cows on the individual treatments. Regression curves indicated that after wk 2 postpartum (14 d) cows injected with 5 mg bST/d both prepartum and postpartum (treatment IV) had greater DMI than any of the three other treatment groups (figure 3-1). Heterogeneity tests of these curves indicated that the

Table 3-7. Least Squares Means and SE for DMI and Energy Status of Holstein Cows During Early Lactation (0-65 d).

	Treatment [1]										
	I		II		III		IV				
No. of Animals	11		12		12		11		Contrast		
Source	Mean	±SE	Mean	± SE	Mean	±SE	Mean	± SE	Pretrt	PPtrt	Pretrt*PPtrt
DMI (kg/d)	20.21	0.15	19.97	0.14	19.08	0.14	23.69	0.15	NS	†	NS
DMI (%BW)	3.5	0.02	3.30	0.02	3.20	0.02	3.70	0.02	NS	†	NS
NE_L Intake (Mcal/d)	31.93	0.23	31.74	0.23	30.20	0.22	36.92	0.24	---	---	---
BW (kg)	608.29	0.51	608.21	0.46	594.85	0.45	633.93	0.49	NS	**	**
BCS	3.08	0.01	3.16	0.00	3.01	0.01	3.25	0.01	NS	**	**
BW Change (kg/d) (BWC)	-0.94	0.12	-1.20	0.12	-0.92	0.12	-0.53	0.12	---	---	---
NE_{BWC} (Mcal/d)	4.46	0.62	5.78	0.60	4.35	0.59	2.44	0.62	---	---	---
Milk Energy (Mcal/d)	25.65	0.22	26.53	0.22	25.47	0.21	29.30	0.23	---	---	---
Energy Balance (Mcal/d)	-7.50	0.72	-10.87	0.70	-9.82	0.69	-6.03	0.72	NS	NS	NS

[1]Treatment (Prepartum, Postpartum); Treatment I= 0,0 mg bST/d; II=0,5 mg bST/d; III=5,0 mg bST/d; IV=5,5 mg bST/d.
NS=Not significant
† $P < 0.1$
** $P < .01$

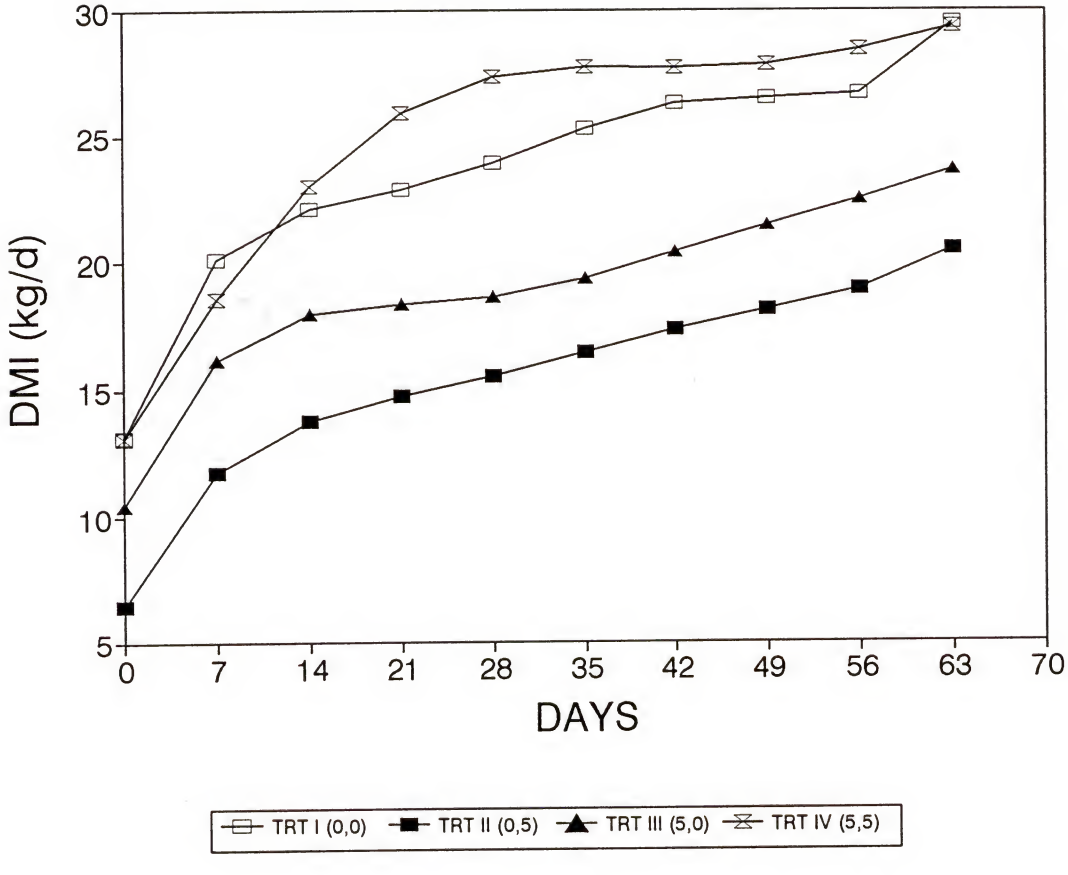

Figure 3-1. Regressions Depicting Dry Matter Intake of Holstein Cows During Early Lactation.

regression curves were not parallel. Results presented in table 3-7 showed that, as a result of greater DMI of prepartum and postpartum bST-treated cows, the NE_L intake also was greater for these cows. Therefore, even though the NE content of milk produced daily was greater for cows in this treatment group because of their higher milk yield (kg/d), changes in BW were less pronounced and thus, milk production likely was supported to a greater degree by feed intake than by tissue mobilization to provide needed the nutrients and energy.

Rate of increase of DMI (kg/d) during early lactation may be the primary determinant of energy intake and balance. Results of this experiment for the first 9 wk after calving agreed with those of Simmons et al. (1994). Although they found DMI tended to decrease 2.1 kg/d for cows injected with 5 and 14 mg bST/d during the prepartum period, the DMI decreased 1.9 kg/d more for cows injected with 14 mg/d. They also reported that after parturition DMI of bST-treated cows increased by about 3 kg/d and no differences among groups were detected. Higher doses of bST apparently exacerbated the decrease in DMI. In other studies it has been reported that in postpartum bST-treated cows there was an increase in DMI after several weeks of injections (Chalupa and Galligan, 1989; McGuffey and Wilkinson, 1991; Salfer et al., 1994) and the increase was dose-dependent; it increased as dose injected increased (Hartnell et al., 1991). In contrast, others have reported no increases in DMI or in feed efficiency (Gallo and Block, 1990a, b) of cows injected with bST during early lactation (Schneider et al., 1990) or during mid to late lactation (Lough et al., 1988; Lucy et al., 1993; Remond et al., 1991). Still others have reported that cows treated with bST during the previous lactation

had higher postpartum DMI during the subsequent lactation (Bauman et al., 1985; Hemken et al., 1991; Lean et al., 1991).

Increase in DMI following injections of bST either prepartum or postpartum may be due to differences in metabolism after parturition when they were treated with bST both prepartum and postpartum. Somatotropin is a homeorhetic controller that directs and shifts partitioning of nutrients, and coordinates the metabolism of various organs and tissues. These coordinated responses in specific tissues involves the metabolism of all nutrients and thus, carbohydrates, lipids, proteins, and minerals are affected (Bauman, 1992). In ruminants, it has been reported that ST increases protein synthesis in muscle without affecting protein degradation (Pell and Bates, 1987; Simmons et al., 1994). Additionally, effects of ST on lipid metabolism depend upon the energy balance of the cows. In cows that are in positive energy balance, ST reduces the synthesis and deposition of lipids in adipose tissue; this accommodates the increase in nutrient utilization for milk production or lean tissue accretion. Therefore, actions of ST involve decreases in activities of lipogenic enzymes, decreases in glucose uptake and oxidation, and decreases in the sensitivity of adipose tissue to INS by reducing number of INS receptors in adipocytes (Bauman and Elliot, 1983; Bauman and Vernon, 1993; Vernon and Taylor, 1988), and by altering the INS uptake into the cell and its degradation (McNamara, 1995). The net result of these changes is a reduction in INS stimulated conversion of NE consumed to a stored form of energy as lipids in adipose tissue via lipogenesis. In cows that are in negative energy balance, ST increases the responsiveness of adipose tissue to β-adrenergic agents by altering number or binding of β-receptors or

by altering responsiveness of adipocytes to adenosine thereby increasing lipolysis (Bauman and Vernon, 1993; Bernal Santos, 1982; McNamara, 1995; Vernon et al., 1991b). Thus, in bST-treated cows that are in negative energy balance the additional energy needed for milk production or lean tissue accretion may be derived largely from increased lipid mobilization (Sechen et al., 1990). In the current study all cows were in positive energy status before parturition, the time when injections of bST started. Even though at calving cows had reduced their DMI by about 35%, they still were in positive energy status (table 3-7). Thus, it may be assumed that NEFA concentrations in plasma were not yet increased, or at least not greatly increased. If this were true then it can be speculated that bST treatment before parturition did not increase lipolysis. If true, then prepartum treatment with bST would not have decreased the replenishment of subcutaneous body fat reserves before parturition, nor given rise to increased blood lipid levels that could lead to excessive fat deposition in liver and the consequent fatty liver syndrome. Moreover, an increase in protein reserves also may have occurred. This would be important because at parturition, with the onset of milk production, dairy cows mobilize both body protein and body fat to support milk production, especially during early lactation. Therefore, after parturition all cows in the current study, and likely all high producing lactating cows, enter a period of negative energy balance. Thus, lipid mobilization likely occurred, although not measured directly. As a general response to prepartum and postpartum injections of bST, it is likely the cows responded by increasing DMI slightly, or more correctly, by attenuating the amount of DMI decrease during early lactation and then stimulated DMI during later lactation. Other effects likely to occur

were changes to increase hepatic gluconeogenesis from propionate and dietary amino acids to supply energy provided as glucose to support increased milk production (Chilliard, 1989), and to provoke a decrease in INS activity and thus, decrease lipogenesis and probably to a lesser extent to increase β-adrenergic action with consequent lipolysis (McNamara, 1988, 1995). These changes and interpretation are supported by literature cited and by measures obtained during the current study.

Although analyses of variance detected a significant ($P < 0.1038$) interaction between prepartum and postpartum treatments (PRETRT*PPTRT) for energy status, orthogonal contrast among the four treatment means indicated that they did not differ (table 3-7). Nevertheless, prepartum and postpartum bST-treated cows had a less pronounced negative energy status (-6.03 Mcal/d) than cows treated prepartum but not treated postpartum (-9.82 Mcal/d), and than cows not treated prepartum but either treated (-10.87 Mcal/d) or not treated (-7.50 Mcal/d) postpartum. For all treatments, the negative energy status observed during the early postpartum period agreed with results seen for untreated cows and is the expected response in high producing postpartum cows. Regression curves depicting the effect of PRETRT*PPTRT on energy balance during the postpartum period are in figure 3-2. These curves generated using regression coefficients indicated that cows treated with bST during both prepartum and postpartum periods reached neutral energy status (intake = outflow) by wk 7 (d 49) and thereafter tended to remain in positive status. Cows not treated before calving but treated after calving also reached neutral energy status by wk 7, but thereafter they tended to remain in neutral status or to be slightly negative. Additionally, cows not treated with bST either before or

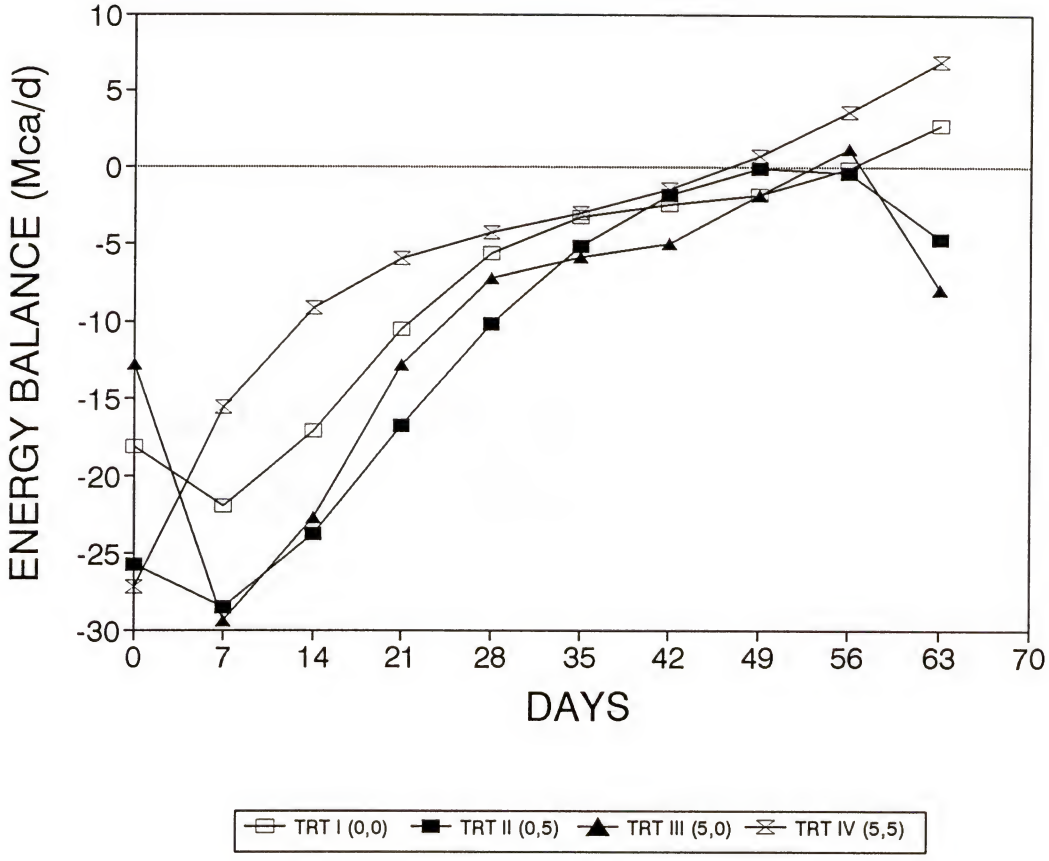

Figure 3-2. Energy Status (NE_L) of Holstein Cows During Early Lactation.

after calving and those treated before calving but not treated after calving reached neutral energy status one week later. These four regression curves that depict these changes were found not to be parallel by test of heterogeneity. The less negative energy status during the postpartum period observed for cows treated with bST both prepartum and postpartum may be explained by the fact that these cows had greater DMI. Therefore, more energy from feed was available to support lactation. During the period of bST injections during the postpartum period, milk yield increases and adaptations in metabolism are of crucial importance. Mammary gland uptake of all precursors needed for milk synthesis increases, whereas metabolism of other tissues is altered to modify and/or conserve the use of these precursors that are critical for milk synthesis (e.g., alternate energy sources are used by extra-mammary tissues). For example, glucose production by the liver increases, whereas its oxidation by body tissues decreases. Clearly, this would favor synthesis of milk because glucose is indispensable as an energy source and it also provides the carbon skeletons for synthesis of milk constituents (e.g., lactose, glycerol). Additionally, lipid metabolism is altered with either reduced lipogenesis or increased lipolysis depending upon the energy status of the cows (Bauman, 1992). In the current study, cows treated prepartum and postpartum with bST, as well as cows from other treatment groups treated or untreated during these periods, had negative energy status after parturition. Therefore, lipolysis likely increased at parturition to provide energy for lactogenesis and to support milk production in all cows and they continued to utilize body fat reserves during early lactation. However, the prepartum and postpartum bST-treated cows that showed greater increases in DMI after wk 2 postpartum

were able to recover to a positive energy status faster and reached positive energy status by wk 7; they also produced numerically greater, but not significantly greater, quantities of milk. This suggests that the utilization of body fat reserves was increased to a lesser extent for this group of cows relative to milk production level. However, tissue utilization of NEFA released by lipolysis, as a metabolic fuel, may have facilitated the reduction in tissue oxidation of glucose which would result in greater availability of glucose for milk production.

Trend for the effects of the interaction PRETRT*PPTRT for BW and BCS were similar to that described for DMI. Cows treated with 5 mg/d of bST before and after calving lost less BW (633.9 vs. 594.9 kg) during the lactation period and had higher BCS (3.25 vs 3.01) than cows also treated before, but not treated after calving, and than cows not treated prepartum and treated or not treated with bST postpartum (633.9 vs. 608.3 and 608.2 kg; 3.25 vs. 3.08 and 3.16 BCS). Orthogonal contrasts indicated that the effect of the interaction PRETRT*PPTRT on BW and BCS was due to PPTRT ($P < 0.01$).

Coefficients from regression analyses were used to plot regression curves depicting the interaction effect of PRETRT*PPTRT on BW (figure 3-3) and BCS (figure 3-4). Curves for BW were not parallel as indicated by the test for heterogeneity. They indicated that the amount of BW loss for cows treated with bST both prepartum and postpartum was significantly less than that of cows for the other three treatment groups. Thus, there was a positive effect of bST injections during both the prepartum and postpartum periods. Cows treated with bST before but not after calving had the greatest BW loss; BW did not begin to increase again until after wk 7 of lactation. Contrary to

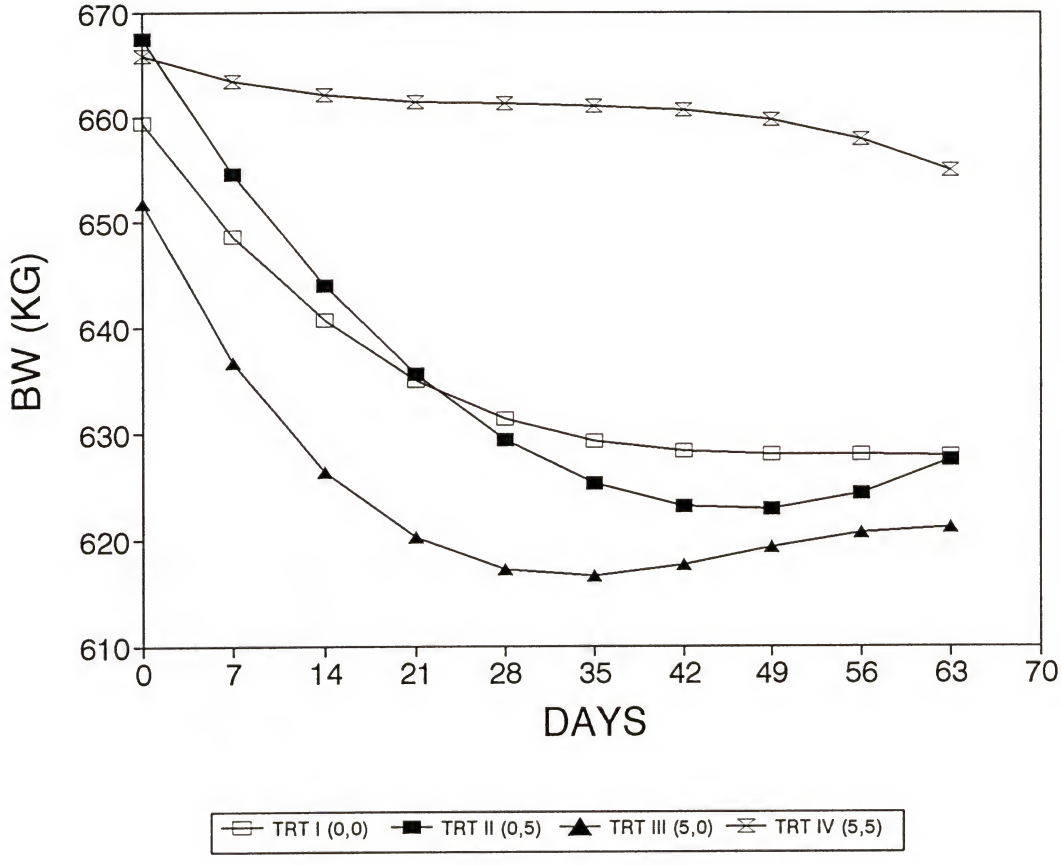

Figure 3-3. Regressions Depicting Body Weight Changes of Holstein Cows During Early Lactation.

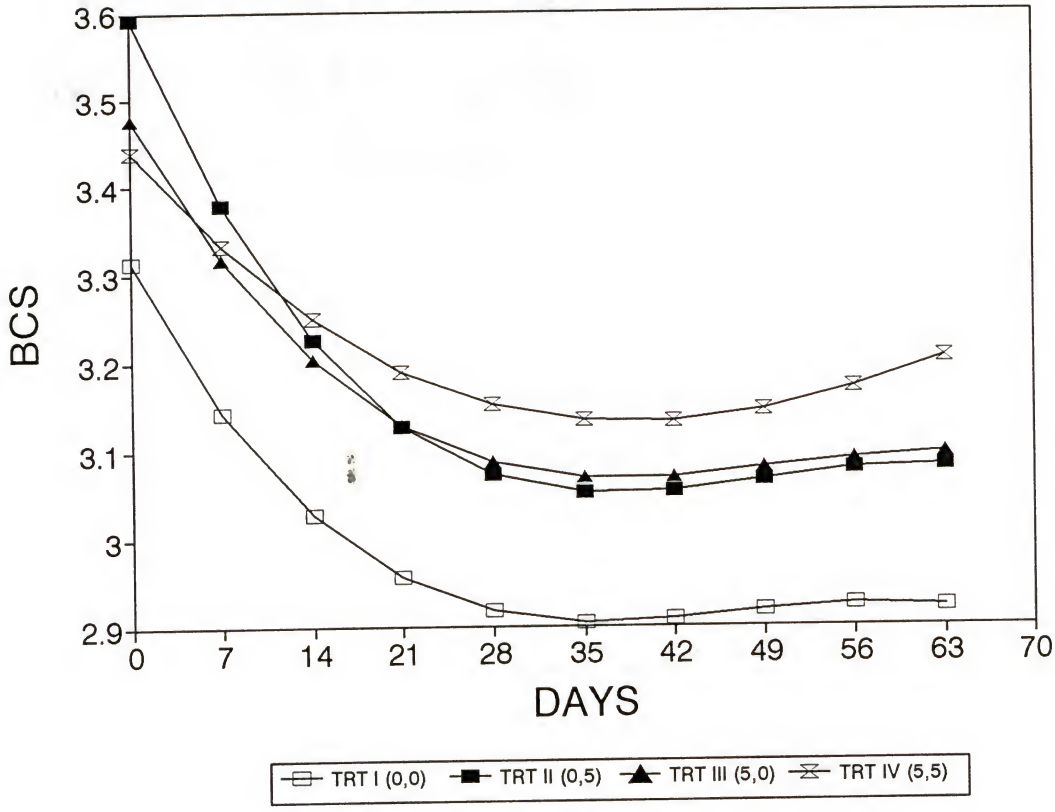

Figure 3-4. Regressions Depicting BCS Changes of Holstein Cows During Early Lactation.

that, for cows not injected at all with bST the BW loss was similar during the first 3 wk of lactation but recovery started earlier, and for cows injected with bST only after parturition the BW loss also was greater than that of cows not injected, and they did not start to increase until after wk 8 postpartum. Changes in BCS followed a similar pattern as BW, as would be expected. Cows in all four treatment groups lost BCS from calving to wk 5 of lactation; however, prepartum and postpartum bST- treated cows started to recover their BW faster and by wk 9 their BCS was higher than for any of the other three treatment groups.

Interestingly, cows not treated with bST prepartum had the greatest loss in BCS and did not start recovery until wk 8 postpartum, thus indicating greater mobilization of adipose tissue to support milk production. Results of the current study agreed with previous findings (Kertz et al., 1991; Staples et al., 1990). However, recuperation of BW and BCS started earlier than that reported previously (Pedron et al., 1993). Additionally, cows with higher BCS at calving (not treated before calving but treated after calving; BCS = 3.6) had reduced BCS and BW for a longer period of time after parturition and started recuperation later than cows with BCS between 3.5 to 3.4. This agreed with results reported by Ruegg and Milton (1995). Results observed agreed with the fact that over-conditioned cows are more likely to have poor appetites postpartum (Fronk et al., 1980; Garnsworthy and Jones, 1987; Garnsworthy and Topps, 1982; Holter et al., 1990). Postpartum DMI has been associated with prepartum DMI; over-conditioned cows consumed less DMI (Emery, 1993; Grummer, 1995).

Variable responses in BCS and BW recuperation after calving have been reported as a consequence of bST treatment. Slower increase in BCS was reported for cows treated with bST from either 9 to 39 or 20 to 36 wk postpartum (Remond et al., 1991; Salfer et al., 1994; Stanisiewski et al., 1992; Tessmann et al., 1991). In the current study, bST-treated cows (before and after parturition) recovered BCS and BW more rapidly than cows not injected with bST. This agreed with previous reports (Dahl et al., 1991; Chilliard et al., 1991) but did differ with results of Simmons et al. (1994) who reported no effect of bST on BCS or BW. Results of the current study likely reflect the fact that there were greater increases in the consumption of DM by cows being treated with 5-8 mg bST/d both prepartum and postpartum. Although bST-treated cows had numerically greater increases in milk yield, it seems that a greater amount of the energy required had been supplied from the increased DMI (energy intake) of these cows. Thus, lipid mobilization, and consequently energy derived from tissue mobilized and reflected by BWC and BCS, was less for these bST-treated cows.

Although analyses of variance for specific days postpartum (0, 1, 3, 7, 14 and 21) did not show differences among treatments or significant interactions, it is noteworthy to describe response to the effect of PRETRT on the dependent variables DMI, BW, BCS and energy status during these specific days during the first 3 wk of lactation.

Least squares means for the effect of PRETRT (bST or not) on DMI, BW, BCS, and energy status during the overall lactation period (0 to 65 d) and specific days are in table 3-8. Although differences between prepartum bST-treated and not treated cows were not observed for these measures, on day 1 after calving both groups of cows had

Table 3-8. Least Squares Means and SE of DMI, BW, BCS and Energy Status of Holstein Cows During the Transition Period from Calving to d 21 Postpartum.

	Overall	SE	d1	SE	d3	SE	d7	SE	d14	SE	d21	SE
DMI (kg)												
bST[1]	21.06	± 0.26	12.89	± 0.27	12.95	± 0.27	15.58	± 0.25	19.78	± 0.26	21.55	± 0.25
No bST	20.34	± 0.26	11.90	± 0.26	14.99	± 0.26	16.94	± 0.26	18.91	± 0.26	18.63	± 0.24
BW (kg)												
bST	614.40	± 0.64	657.45	± 0.88	649.61	± 0.88	636.15	± 0.88	625.08	± 0.45	614.61	± 0.67
No bST	608.25	± 0.63	661.48	± 0.87	650.96	± 0.88	634.47	± 0.87	617.84	± 0.44	603.31	± 0.64
BCS												
bST	3.13	± 0.01	3.45	± 0.01	3.41	± 0.01	3.30	± 0.01	3.23	± 0.01	3.18	± 0.01
No bST	3.12	± 0.01	3.43	± 0.01	3.56	± 0.01	3.26	± 0.01	3.14	± 0.01	3.00	± 0.01
Energy Status ($Mcal \cdot d^{-1}$)												
bST	-7.93	± 1.09	-34.86	± 1.98	-32.33	± 1.97	-23.07	± 1.98	-11.40	± 0.84	-14.42	± 1.03
No bST	-9.19	± 1.08	-41.59	± 1.96	-30.97	± 1.96	-28.51	± 1.96	-18.79	± 0.83	-17.44	± 1.07

[1]5 mg bST/d starting 3 wk before parturition.

increased DMI which averaged 3 kg/d more than that on day of calving. Thus, DMI on d 1 was similar to that observed on d -3 prepartum but still was only 87 % of the DMI on d -7 before parturition. These results agreed with the increase reported (1.5 to 3 kg DMI) for the first 3 wk after calving (Bertics et al., 1992; Nocek et al., 1983; Kertz et al., 1991). However on d 1 postpartum, as expected, the injected and not injected groups of cows were in negative energy status, and as indicated already, this has been associated with the high energy demands and uptake of glucose for lactogenesis and milk synthesis in the mammary gland (Davis et al., 1979). Additionally, it has been associated with the stress of parturition and weight loss associated with uterine emptying (calving). Interestingly, on d 3 postpartum the DMI for both pretreatment groups of cows were comparable to the DMI on d -7 (prepartum) and thus, also similar to the DMI on d -21 prepartum. However, even though prepartum bST-treated cows consumed 2 kg less DMI than cows not treated (12.95 vs. 14.99 kg/d), the negative energy status of bST-treated cows was similar to that of not treated cows. Although the decrease in BCS for cows that were not treated was more noticeable than for bST-treated cows, the BWC was similar for both. On the other hand, on d +7 after calving, although DMI increased for both pretreatment groups, DMI of prepartum bST-treated cows was 1.4 kg/d less than DMI of not treated cows, but this latter group lost more BW than prepartum bST-treated cows (32.1 vs.27.5 kg) from parturition up to d +7 of lactation. Thus, the energy status for cows on this day of lactation was more negative than the energy status of prepartum bST-treated cows. Moreover, on d + 14 both prepartum treatment groups of cows had increased their DMI by 40% more than the DMI during the prepartum period (either d -21 or -7), but increase in DMI of prepartum bST-treated cows was slightly greater than that of not treated cows. Furthermore, BW loss for the cows not treated prepartum with bST was

greater (48.8 vs. 38.5 kg) and the loss in BCS also was greater (3.14 vs. 3.23) than for prepartum bST-treated cows. Thus, the negative energy status of prepartum bST-treated cows at d + 14 postpartum was less pronounced than the negative energy status for not treated cows (-11.40 vs. -18.79 Mcal/d). Additionally, on d + 21 postpartum the DMI of prepartum bST-treated cows was 2.92 kg greater than for cows not treated. Changes in BW and BCS loss were greater for cows not treated prepartum and again, this group had more pronounced negative energy status (-17.44 vs. 14.42 Mcal/d for bST-treated cows).

Milk Yield, 4% FCM and Milk Constituents

Least squares analyses of variance for milk and 4% FCM yield are in table 3-9. Results detected no significant differences for main effects or for any of the two- or three-factor interactions. Conversely, least squares analyses of variance for milk constituents (table 3-10) detected significant ($P < 0.0535$) differences for the interaction PRETRT*PPTRT for SNF %. Significant differences also were observed for the main effect of diet ($P < 0.0403$) and PPTRT ($P < 0.0121$) for FAT %, whereas no significant effects were detected for CP %.

Main objectives of this research were to study the effects of low doses of bST and nutritional status of the cows during prepartum and early postpartum periods to determine if these treatments were beneficial. To critically evaluate the effects of these treatments on milk production and incidences of various metabolic diseases would have required a much greater number of cows in each of the treatment groups. Even though no significant differences for milk production measures were detected by analyses of variance, it is important to describe means for

Table 3-9. Least Squares Analyses of Variance for Milk Yield and 4% FCM Yield of Holstein Cows During Early Lactation (0-65 d).

Source		Milk Yield			4% FCM		
	df	MS	F	P>F	MS	F	P>F
Pretrt[1]	1	610.56	0.25	0.6223	1295.47	0.51	0.4809
Diet	1	12.35	0.00	0.9441	1301.76	0.51	0.4798
Pretrt * Diet	1	3502.74	1.41	0.2416	2923.96	1.14	0.2916
PPtrt[2]	1	321.36	0.13	0.7206	2868.04	1.12	0.2962
Pretrt * PPtrt	1	6415.28	2.59	0.1157	4418.20	1.73	0.1965
Diet * PPtrt	1	2357.92	0.95	0.3353	2990.38	1.17	0.2862
Pretrt * Diet * PPtrt	1	69.54	0.03	0.8678	85.68	0.03	0.8557
Cow (Pretrt * Diet * PPtrt)	38	2475.45	81.32	0.0001	2555.98	62.37	0.0001
Days (D)[3]	1	40734.21	1338.14	0.0001	16179.08	394.74	0.0001
D * D	1	18177.54	597.14	0.0001	11265.30	274.88	0.0001
D * D * D	1	4760.46	156.38	0.0001	4145.88	101.16	0.0001
D * D * D * D	1	1027.48	33.75	0.0001	1216.07	29.67	0.0001
D * D * D * D * D	1	484.15	15.90	0.0001	510.72	12.46	0.0004
Error	2894	22.46			31.27		

[1]Prepartum treatment.
[2]Postpartum treatment.
[3]Type I Sums of Squares for all D terms; others are Type III.

Table 3-10. Least Squares Analyses of Variance for Milk Constituents of Holstein Cows During Early Lactation (0-65 d).

		SNF[1] %		CP[2] %		Fat %	
Source	df	MS	PR > F	MS	PR > F	MS	PR > F
Pretrt[3]	1	0.03	0.9699	0.11	0.8545	5.17	0.4467
Diet	1	24.99	0.2907	0.27	0.7726	39.40	0.0403
Pretrt * Diet	1	30.07	0.2472	2.36	0.3929	0.35	0.8415
PPtrt[4]	1	12.23	0.4580	0.74	0.6308	60.64	0.0121
Pretrt * PPtrt	1	86.47	0.0535	5.00	0.2156	6.12	0.4076
Diet * PPtrt	1	2.28	0.7476	0.19	0.8081	1.30	0.7022
Pretrt * Diet * PPtrt	1	8.53	0.5349	1.17	0.5469	0.89	0.7519
Cow (Pretrt * Diet * Pptrt)	38	21.77	0.0001	3.15	0.0001	8.74	0.0001
Days (D)[5]	1	319.87	0.0001	88.38	0.0001	217.75	0.0001
D * D	1	57.25	0.0001	40.18	0.0001	55.56	0.0001
D * D * D	1	33.83	0.0003	6.02	0.0001	8.97	0.0001
D * D * D * D	1	1.68	0.4232	1.50	0.0001	0.98	0.0732
D * D * D * D * D	1	5.97	0.1312	0.26	0.0137	0.29	0.3307
Error	2947	2.62		0.04		0.31	

[1]Solids-Not-Fat. [2]Crude Protein. [3]Prepartum treatment. [4]Postpartum treatment.
[5]Type I Sums of Squares for all D terms; others are type III.

the interaction PRETRT*PPTRT to evaluate whether bST treatments before and/or after parturition had large and significant effects on any of the milk or milk constituent measures.

Least squares means for milk yield, 4% FCM yield, and milk constituents are in table 3-11. Results indicated that during the overall lactation period (0 to 65 d) cows that were injected prepartum and postpartum with bST had greater milk yields and 4% FCM yields (kg/d) than cows that were injected prepartum but not postpartum (39.27 vs. 35.64 and 39.34 vs. 34.89), and greater than cows not injected prepartum but injected postpartum (39.27 vs. 35.39 and 39.34 vs. 35.55). However, when prepartum and postpartum bST-treated cows were compared to those not injected at all, differences in milk yields (39.27 vs. 37.69 kg/d) and in 4% FCM yields (39.34 vs. 36.03 kg/d) were less pronounced. Observed differences likely are the result of action of bST on metabolism of these early lactation cows.

Regression analyses were performed for milk and 4% FCM yields to describe the trends in production over the 65 d lactation. Quintic order regression curves for milk (figure 3-5) and 4% FCM (figure 3-6) indicated that after 2-wk in lactation the cows that had been injected with low doses of bST during both the prepartum and postpartum periods had greater and sustained increase in production compared to cows in the other three treatment groups. The greater and sustained increase in production agreed with the results for DMI because the same treatment group also had greater DMI at about the same time. This supports the view that the energy consumed was used to synthesize milk and

Table 3-11. Least Squares Means and SE for Milk Yield, 4% FCM Yield, and Milk Components of Holstein Cows During Early Lactation (0-65 d).

Treatments[1]	I		II		III		IV				
No. of Animals	11		12		12		11		Contrast		
Source	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Pretrt[2]	PPtrt[3]	Pretrt*PPtrt
Milk Yield (kg/d)	37.69	0.21	35.39	0.20	35.64	0.20	39.27	0.21	NS	NS	NS
4% FCM (kg/d	36.03	0.24	35.55	0.23	34.89	0.23	39.34	0.24	NS	NS	NS
Fat (%)	3.71	0.02	4.09	0.02	3.89	0.02	4.08	0.02	NS	†	**
Fat (kg/d)	1.40	0.01	1.43	0.01	1.38	0.01	1.58	0.01	NS	NS	NS
Protein (%)	2.90	0.01	3.02	0.01	2.97	0.01	2.92	0.01	NS	NS	**
Protein (kg/d)	1.08	0.01	1.04	0.01	1.04	0.01	1.13	0.01	NS	NS	NS
SNF (%)	8.85	0.06	9.32	0.06	9.19	0.06	8.97	0.06	NS	NS	**
SNF (kg/d)	3.32	0.03	3.25	0.03	3.25	0.03	3.51	0.03	NS	NS	NS

[1]Treatment (Prepartum, Postpartum); Treatment I= 0,0 mg bST/d; II=0,5 mg bST/d; III=5,0 mg bST/d; IV=5,5 mg bST/d.
[2]Prepartum treatments.
[3]Postpartum treatments.
NS=Not significant
† P<0.1
** P<0.01

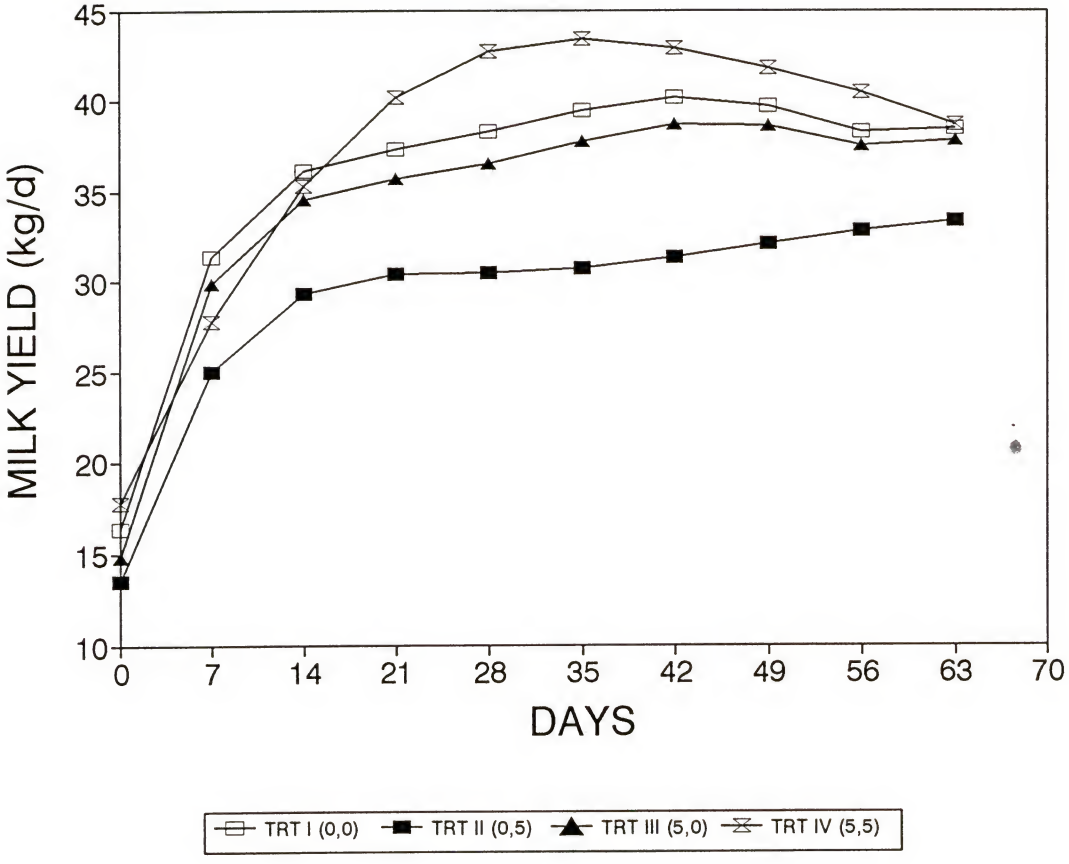

Figure 3-5. Regressions depicting Milk Production of Holstein Cows During Early Lactation.

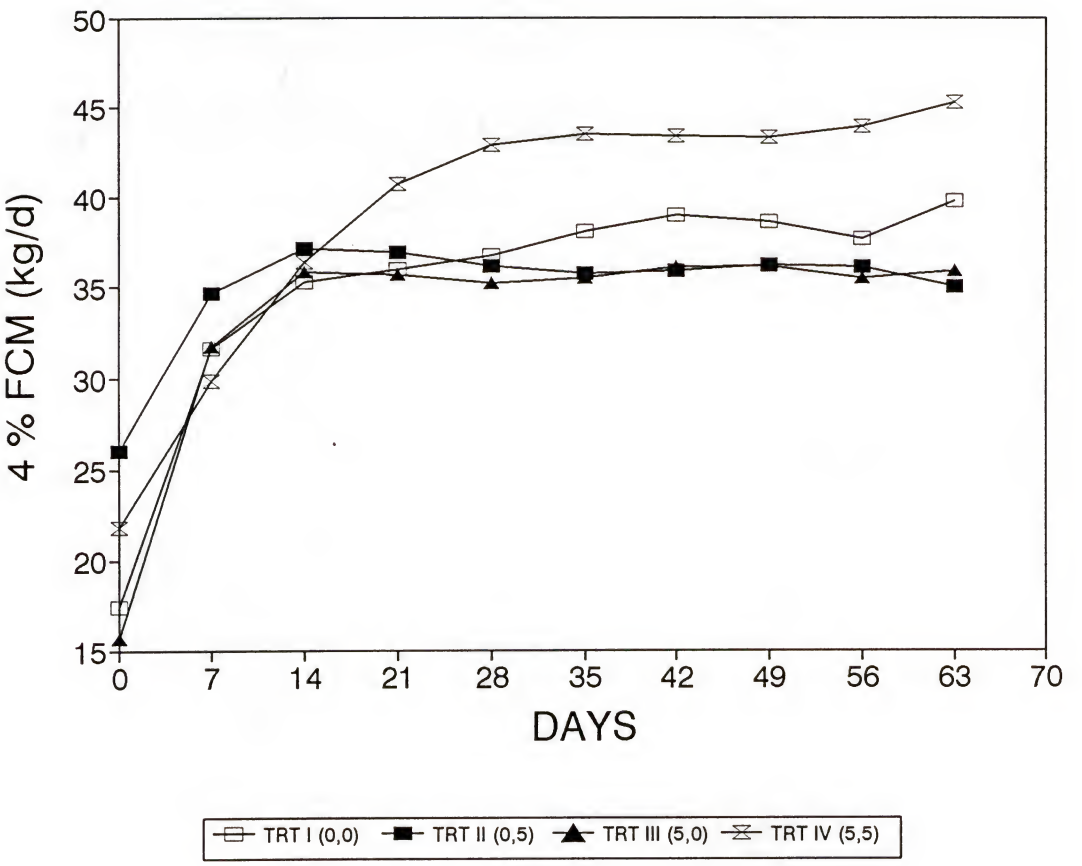

Figure 3-6. Regressions depicting 4% FCM Production of Holstein Cows During Early lactation.

that mobilization of nutrients, even if delayed somewhat after calving, did not limit milk production to the same degree as it may have in the other treatment groups. At the same time, this group had less body weight loss and this supports the view that maintaining a high level of feed intake around the time of calving and early in the lactation is important for milk production and energy status of the treated cows.

Somatotropin is considered a homeorhetic controller that exhibits regulatory effects on metabolism and consequently, provides control over partitioning of absorbed nutrients and those mobilized from body stores for growth and lactation. Changes in tissue metabolism involve both direct effects on some tissues such as liver and adipose tissue, and indirect effects mediated by the ST-dependent somatomedins (IGFs) for other tissues such as mammary gland (Bauman, 1992). Cows treated with bST had increased IGF-I concentrations in plasma and milk during both early and late lactation. However, milk IGF-I concentration was greater in the early postpartum period than that of bST-treated cows at later stages of lactation (Bauman, 1992; Dahl et al., 1993; Prosser et al., 1989; Zhao et al., 1994). High concentrations of IGF-I during the late dry period are associated with the action of IGF-I as a local mediator of mammary epithelial growth and development (Vega et al, 1991). IGF-I is increased by exogenous administration of ST (McShane et al., 1989; Crooker et al., 1990) and can stimulate multiple cellular responses related to tissue growth, including synthesis of DNA and cellular protein (Clemmons and Underwood, 1991; Purup et al., 1995). Receptors for IGF-I have been demonstrated in mammary tissue of cows. These receptors are involved in the IGF-I-mediated actions of ST in the mammary gland (Dehoff et al., 1988). Moreover, mammary gland IGF receptor

numbers change with the physiological state of the tissue. For example, IGF-I receptor numbers decline during the prepartum period, and increase as much as 75% during lactogenesis; this increase is associated with increased binding of IGF-I to mammary microsomes, and finally, receptor numbers decline during the postpartum period (Dehoff et al., 1988). The changes observed support the theory that IGF-I plays an important role in modulating the metabolic activity of bovine mammary gland by acting directly on normal mammary epithelial cells to stimulate DNA synthesis and to increase cell numbers.

Observed differences due to the effect of PPTRT on FAT% are associated with the direct actions of ST to regulate tissue metabolism, especially that affecting adipose tissue metabolism with the consequent increase in lipid mobilization and increased uptake of NEFA by the mammary gland and other tissues. Differences observed for the main effect of diet on FAT % also may be due to differences in the fat content of the diets. TMR diet that contained WCS provided an added source of fat and cows that were fed this TMR postpartum did produce milk with greater FAT%; chemical analyses for this diet showed high content of fat (table 3-3). Therefore, there was greater availability of TG for mammary gland uptake and synthesis of milk fat. These results agreed with other reports where increasing the fat content of the diet improved milk production and also slightly altered the composition of the milk fat.

Efficiency of milk production and the apparent efficiency of 4 % FCM production were calculated and the gross efficiency and corrected efficiency for 4% FCM production are in table 3-12. Apparent efficiency of milk production (4% FCM/DMI) was greater for

cows injected with 5 mg/d of bST during the prepartum period but not injected after parturition (1.83 kg), followed by cows not injected during the prepartum period, but injected after parturition (1.78 kg); efficiency of cows not injected at any time was 1.74 kg and was least for cows injected during both the prepartum and postpartum periods. Gross efficiency of milk production (4% FCM/NEI) followed similar trends; efficiencies were 1.12, 1.12, 1.16 and 1.08 for cows not injected at any time, cows not injected during the prepartum period but injected after parturition, cows injected before parturition but not injected thereafter, and for cows injected during both the prepartum and postpartum periods. Because of the body weight loss (BWC) during the early lactation period, efficiency of milk production derived from body tissue was calculated. Cows with the greatest BWC produced more milk derived from energy of tissue origin. Thus, cows injected with bST during both prepartum and postpartum periods that had lowest BWC had less milk derived from tissue energy (3.26 kg) compared to cows not injected during prepartum but injected after parturition that had large BWC (7.73 kg), cows not injected at any time (5.96 kg), and cows injected before parturition but not injected during postpartum period (5.82 kg). Consequently, efficiency of production of 4% FCM was greater for cows injected with bST during both the prepartum and postpartum periods (1.57 vs. 1.45, 1.39 and 1.52 kg/d) compared to the other three treatment groups.

Table 3-12. Least Squares Means and SE for Milk Yield, 4% FCM Milk Yield, and Efficiency of Milk Production of Holstein Cows During Early Lactation (0-65 d).

	Treatments[1]			
	I (n=11)	II (n=12)	III (n=12)	IV (n=11)
Measures				
4% FCM[2] (kg/d)	36.06	35.55	34.89	39.34
DMI[3] (kg/d)	20.71	19.97	19.08	23.02
NE_L Intake (NEI; Mcald·d^{-1})	32.78	31.74	30.20	36.42
Milk Energy (Mcal·d^{-1})	25.65	26.53	25.47	29.30
BWC[4] (kg/d)	-0.94	-1.20	-0.92	-0.53
NE_{BWC} (Mcal·d^{-1})	4.46	5.78	4.35	2.44
Apparent Efficiency (FCM/DMI; kg)	1.74	1.78	1.83	1.71
Gross Efficiency (FCM/NEI; kg/Mcal)	1.12	1.12	1.16	1.08
Estimated 4% FCM from BWC (kg)	5.96	7.73	5.82	3.26
Corrected 4% FCM from Feed[5] (kg/d)	30.07	27.82	29.07	36.08
Corrected efficiency of 4% FCM[6] (kg/d)	1.45	1.39	1.52	1.57

[1]Treatment (Prepartum, Postpartum); Treatment I= 0,0 mg bST/d; II=0,5 mg bST/d; III=5,0 mg bST/d; IV=5,5 mg bST/d; numbers of animals in parentheses.
[2]Fat Corrected Milk (4%). [3]Dry Matter Intake. [4]Body Weight Change. [5]4% FCM Produced - 4% FCM from energy from BWC. [6]Corrected 4% FCM from Feed÷DMI.

Conclusions

From the results of this study it can be concluded that use of low doses of bST during the prepartum portion of the transition period caused no negative effects on the ability of the treated cows to replenish their reserves of body tissues over this short-time period. Cows that were treated or not treated with bST during this time appeared equally capable of tissue mobilization to provide energy and substrates for milk synthesis during the lactation that followed.

Injections of low doses of bST during the early postpartum period increased rate of increase of DMI after parturition and, thus, greater amount of nutrients and energy were ingested to support the lactation phase. Furthermore, greater DMI when cows were injected with bST prepartum and postpartum allowed cows to recover BW and BCS at a faster rate and to reach positive energy status in less time during the lactation while they were producing numerically greater, but not significantly greater, amounts of milk and 4% FCM. Finally, less BW loss during the postpartum portion of the transition period and a slightly greater level of milk production during this period resulted in more efficient production of 4% FCM. Although cow numbers were too small to critically evaluate the effects of these treatment combinations on health and calving problems, no apparent differences were noted. It appears that injections of bST could be used during the prepartum or the postpartum periods or both, but indications are that treating cows during both periods had positive effects that will improve efficiency of milk production and perhaps improve overall yields of milk. The latter conclusion must be tested and proven

with a greater numbers of cows over the entire 305-d lactation to ensure that no determental effects occurred during the declining phase of the lactation.

CHAPTER 4
EFFECTS OF NUTRITIONAL STATUS AND PREPARTUM AND POSTPARTUM BST TREATMENT ON METABOLIC HORMONES AND OVARIAN ACTIVITY OF DAIRY COWS DURING EARLY LACTATION

Introduction

Lactation results in a decrease in the rate of fatty acid synthesis in adipose tissue (Vernon and Taylor, 1983). This adaptation occurs to facilitate the preferential use of lipogenic precursors by the mammary gland. Hormones responsible for this adaptation were shown to be PRL and ST (Vernon et al., 1991). Additionally, the hypoinsulinemia of lactation may contribute to decreased fatty acid synthesis. Decreased fatty acid synthesis is due to the reduced activity and total amount of active acetyl-CoA carboxylase enzyme; these changes probably are induced by changes in the serum concentrations of INS and ST.

Nutritional status modifies serum concentrations of ST; metabolites such as glucose, lipids, and amino acids also have been postulated to be secondary controlling factors that modulate ST secretion (Daughaday, 1981). The dynamic fluctuations of plasma hormones and metabolic substrates that occurs during the postprandial and post-absorptive states provides signals to the brain that link metabolic status to the activation of the reproductive system. Collectively, data have demonstrated that under certain

physiological conditions such as lactation, alterations in energy metabolism may influence hypothalamic-pituitary function.

Insulin, Somatotropin and Insulin-like Growth Factor-I

Insulin regulates cellular metabolism in a large number of target tissues and it is considered to be an anabolic agent because it is involved in the stimulation of several synthetic pathways such as for glycogen and protein synthesis. However, catabolic functions also are mediated by INS, including lipolysis, glycogenolysis or protein degradation. Insulin depresses the amount of glucose in circulation by inhibiting gluconeogenesis and glycogenolysis and by stimulating the uptake and utilization of glucose by tissues such as liver, muscle and adipose (Hart et al., 1978). In ruminants, as in monogastric animals, it has been established that a high concentrations of INS in the circulation are necessary to promote the conversion of glucose to glycogen and lipids (Hart et al., 1978). Molecular mechanisms of action of INS in mammary cells have been described to occur as a result of membrane receptor-hormone interactions to induce synthesis of a second messenger cAMP (McCarty and McCarty, 1974; McGuire et al., 1995; Seymour et al., 1988). Hyperinsulinemia generally has been shown to inhibit milk secretion.

Possible mechanisms of action of INS in regulating mammary metabolism include alteration of membrane transport of sugar and amino acids, alteration of hexokinase isoenzyme distribution, direct or indirect regulation of metabolic pathways including cAMP, regulation of tissue enzymes or protein synthesis, and/or control of

redox state or energy charge within the cell (Baldwin and Suchet, 1974). In adipose tissue, INS is the metabolic regulator for fat deposition or mobilization. The glucogenic-INS theory suggests that high propionate production in the rumen leads to increased hepatic rates of gluconeogenesis which, in turn, increases pancreatic release of INS. This results in elevated circulating concentrations of INS which enhances uptake of lipogenic precursors and decreases the release of fatty acids from adipose tissue (Grinari et al., 1997). A number of metabolic effects have been described that result from the increase in circulating INS. Thus, INS would stimulate *de novo* lipogenesis in adipose tissue favoring the use of acetate and β-hydroxy butyrate (BHBA) as carbon sources. In the adipose tissue of ruminants, glucose furnishes reducing equivalents and the glycerol backbone to support TG synthesis (Bauman and Davis, 1975). Additionally, INS stimulates lipoprotein lipase and, therefore, increases uptake and incorporation of fatty acids from circulating TG into lipid reserves. Finally, INS inhibits lipolysis and increases fatty acid reesterification in adipose tissue.

During late pregnancy there is an INS resistance which is manifested as diminished sensitivity of several parameters of whole body glucose utilization to INS and decreased responsiveness of adipose tissue lipolysis and NEFA mobilization to INS (Petterson et al., 1993, 1994). Somatotropin decreased ability of INS to inhibit gluconeogenesis (Sechen et al., 1990) and also decreased lipogenesis by reducing INS receptors in adipocytes or by inhibiting the action of second messenger by blocking its synthesis (Bauman and Elliot, 1983; Bauman and Vernon, 1993; McNamara, 1995; Vernon and Taylor, 1988). Lucy et al. (1991) found that plasma concentrations of INS

after calving and during early lactation depended upon the energy balance, with higher positive energy balance associated with greater plasma concentrations of INS. This demonstrates the importance of INS for the coordination of metabolic processes surrounding nutrient partitioning and utilization. This suggested that INS was one possible modulator of the actions of positive energy balance on follicular function. However, neither supplemental fat nor bST treatment alone had a significant effect on plasma INS concentrations. When cows were fed fat and injected with bST, concentrations of INS were decreased by about 50% (Lough et al., 1988). Nevertheless, INS, cortisol, hemoglobin and hematocrit were not affected by bST treatment, whereas NEFA, ST, and IGF-I were increased significantly (Gallo and Block, 1990b). Treatment with bST during late lactation, the dry period, and the early part of the subsequent lactation resulted in increased INS concentrations during late lactation and dry period, but not during early lactation. This may be due to greater availability of glucose during the dry period and late lactation, when cows were in positive energy balance, but glucose was not being used for the synthesis of milk (Vicini et al., 1991)

Because ST is a homeorhetic controller that shifts the partitioning of nutrients, it is involved in coordination of the metabolism of various organs and tissues. Changes in tissue metabolism involves both direct effects on some tissues and indirect effects on other tissues; changes that are mediated by somatotropin-dependent somatomedins (IGFs) (Bauman, 1992). Plasma concentrations of IGF-I and II are partially under ST control and nutritional status has been shown to play a major role in the regulation of the somatotropic-axis in both monogastric and ruminant species (Breier et al., 1986;

Maes et al, 1983; Phillips and Unterman, 1984). The secretion of hormones from many cell types is regulated by the IGFs. IGF-I directly inhibits ST secretion and this inhibitory effect is potentiated by over-expression of recombinant IGF-I receptors (Jones and Clemmons, 1995). Circulating concentrations of IGF-I generally correlate well with ST secretion during postnatal life. Hepatic expression of IGF-I is responsive primarily to ST. However, about 80% of IGF-1 circulating in blood is bound to 150 kDa-IGFBP, consisting of IGFBP-3 and an acid-labile subunit. Thus, it acts as a storage form of IGF-I. The IGFs are synthesized locally and have potential paracrine and autocrine actions in tissues (Jones and Clemmons, 1995). Tissue IGF-I levels are dependent on ST. IGF-I stimulates peripheral glucose uptake without suppressing hepatic glucose production. IGF-I has no effect on fatty acid concentrations, but it stimulates protein synthesis in ST deficient mice and also total body growth in the ST deficient animal. IGF-I stimulates glucose uptake and glycogen synthesis in INS deficient diabetic rats. Anabolic actions of IGF-I include stimulation of multiplication of many types of cells; promotion of processes related to energy storage, including amino acid transport and protein synthesis in muscle, glucose transport, and formation of lipid and fatty acid synthesis in the liver, and stimulation of processes related to skeletal elongation including synthesis of RNA, DNA, protein and proteoglycan in chondrocytes and cartilage explants. Both hepatic and extrahepatic tissue synthesis of IGF-I may constitute a switch which promotes nutrient utilization for growth related processes involving both endocrine and paracrine mechanisms (Phillips et al., 1990).

Cows in positive energy balance (3.43 Mcal/d) during the first 12 wk postpartum had greater plasma concentrations of IGF-I and greater luteal-phase secretion of P_4 than in cows with negative (-1.69 Mcal/d) energy balance (Spicer et al., 1990). Thus, reduced luteal activity that accompanies negative energy balance may be associated with reduced concentrations of IGF-I in serum. Restriction of DMI in growing steers decreased basal concentrations of IGF-I in blood and abolished the response of IGF-I and IGF-II to exogenous bST (Breier et al., 1988; Ronge and Blum, 1989). Low circulating concentrations of IGF-I during early lactation were associated with low nutrient balances (Ronge et al, 1988; Spicer et al., 1990; Vicini et al., 1991). Response of IGF-I to bST treatment was greater during the dry period when cows were in positive nutrient balance. Bachman et al. (1992) reported significant ($P<0.08$) differences in plasma concentrations of IGF-I in dry cows injected daily with bST beginning 21 d before the expected calving. They also reported that concentrations of ST were 183% greater in treated cows, whereas no differences were observed for other hormones (INS, T_3, T_4, or PRL). Concentrations of IGF-II were not affected by either stage of lactation or restriction of nutrients; however, IGF-II concentrations tended to increase when cows were in positive nutrient balance during the dry period and when cows were fed diets containing an excess of protein and energy during lactation (McGuire et al., 1992b). Treatment of cows with bST increased plasma and milk concentrations of IGF-I during both early and late lactation. However, milk IGF-I concentrations were greater in the early postpartum period than that of bST-treated cows at later stages of lactation (Bauman, 1992; Dahl et al., 1993; Prosser et al., 1989; Zhao et al., 1994).

Concentrations of IGF-I in mammary secretions were 40% greater at parturition for bST-treated cows than for cows fed either 100 or 70% of NRC requirements (Hadsell et al., 1993). High concentrations of IGF-I during late dry period were associated with action of IGF-I acting as a local mediator of mammary epithelial growth and development (Vega et al., 1991).

Prepartum treatment of cows with either 5 or 14 mg bST/d increased and maintained serum ST concentrations at 6.5 and 22.7 ng/ml compared to 1.6 ng/ml for untreated controls. Conversely, IGF-I concentrations were increased initially but were not maintained high as parturition approached and were similar for all groups of cows after parturition. Concentrations of ST and IGF-I in plasma were higher for bST treated cows, whereas concentrations of INS and P_4 were equivalent to that seen in untreated cows (Lucy et al., 1993). Energy balance appeared to be a major regulator of concentrations of IGFBP-3 and responsiveness of IGF-I to exogenous ST before parturition (Simmons et al., 1994). However, IGF-I concentrations were increased during all bST treatment periods, but it was low during early lactation, whereas IGFBP-2 was higher during negative nutrient balance. These results suggested that availability of nutrients may be involved in regulating these proteins (Vicini et al., 1991). Several reports in beef cattle support the idea that concentrations of IGF-I in serum may be an index of nutritional status. In addition to reduced IGF-I secretion, negative energy balance was associated with reduced blood glucose and high NEFA and ketone body concentrations, which suggests insufficient gluconeogenesis and enhanced fat mobilization and ketogenesis occurred during negative energy balance (Spicer et al., 1990).

Somatotropin, IGF-I and Ovarian Activity

IGF-I influenced proliferation and differentiation of granulosa cells *in vitro*. In rats, expression of IGF-I mRNA and the levels of IGF-I in the ovary varied during the estrous cycle; levels were increased between proestrus and estrus (Carlsson et al., 1989). Because of the negative correlation between energy balance and concentrations of circulating IGF-I during early lactation and the role of IGF-I in increasing steroidogenesis, IGF-I has been implicated as a mediator of effects of energy balance on reproduction (McGuire et al., 1992a). Evidence of positive effects of the bST-induced increase of IGF-I on ovarian and luteal function and on follicular growth is accumulating. Low doses of bST may increase reproductive performance. Cows injected with 5 mg bST/d during early lactation returned to first estrous 9 d earlier than control cows, had improved pregnancy rate, and they also had greater first service conception rate (Stanisiewski et al., 1992). bST treatment for two estrus cycles increased serum concentrations of IGF-I and number of small follicles (2 to 5 mm) without affecting concentrations of FSH or LH in serum or the number of medium and large follicles (Gong et al., 1991)

Hormones or growth factors such as ST or IGF-I stimulate ovarian follicular development or function. Increased numbers of small follicles did not result in more class 3 follicles, and therefore, no stimulation of large size follicles was observed with bST treatment (Lucy et al., 1993). However, cows treated with 51.6 or 86.0 mg bST/d during one lactation had longer intervals from calving to conception than untreated cows. Nevertheless, cows treated with bST during the previous lactation, but not during the

second lactation, had significantly shorter intervals from calving to conception (Esteban et al., 1994). Low doses of bST may have increased reproductive performance, but this was not established. Treatment with bST for two estrous cycles increased serum IGF-I concentrations and number of small follicles (2-5 mm) without affecting serum FSH or LH and the number of medium and large follicles (Gong et al., 1991). Treatment with bST increased the number of antral follicles of 2-5 mm in diameter in mature heifers (Gong et al., 1991;1993a). It is likely that the relationships among bST, IGF-I and reproductive efficiency are sensitive to the nutrient and energy status of the animal. It has been suggested that energy (glucose or other gluconeogenic metabolite including amino acids, at least for gluconeogenesis) may affect transcription of IGF-I mRNA, whereas protein (amino acids) may primarily control translation (McGuire et al., 1992a). Feed restriction did not affect IGF-I in blood or its mRNA in liver when lambs were treated with bST (Pell et al.,1991). High energy intake decreased plasma ST, but bST treatment restored ST concentrations in plasma and increased IGF-I secretion from hepatic and non-hepatic tissues.

An increase in energy balance is associated with an increase in concentrations of IGF-I in serum during early lactation. This increase in concentration of IGF-I is associated with increased secretion of P_4 during diestrus of the first and second postpartum estrous cycle, but increased milk yield is associated with decreased concentrations of IGF-I in serum and decreased energy balance (Spicer et al.,1990). Early postpartum treatment of dairy cows (d 14 after calving) with 5 mg bST/d increased FCM production and perhaps reproductive performance (Stanisiewski et al.,

1992). It also increased concentrations of IGF-I in serum. Peak concentrations of P_4 during first estrous cycle that occurred 32 d after parturition were twice as great in cows in positive energy balance as in cows in negative energy balance (Spicer et al, 1990). Low concentrations of INS during early lactation may limit the IGF-I response to rbST. Reduced basal ST concentrations and reduced blood concentrations occurred with high plane of nutrition and restoration of adequate feeding (Leonard and Block, 1997). It appears that nutritional status influences the relationship between ovarian and systemic levels of INS and IGF-I.

Insulin and IGF-I appear to stimulate granulosa cell mitosis and this mitogenic effect of IGF-I is enhanced by FSH and LH from small but not large follicles (Gong et al., 1993b). INS stimulated E_2 production by the bovine granulosa cells, whereas IGF-I and II inhibited INS-induced production of E_2 by granulosa cells of both small and large bovine follicles (Spicer et al., 1993; Spicer and Echternkamp, 1995). INS treatment increased the concentrations of E_2 in follicular fluid and a change in follicular fluid concentrations of IGF-I.

INS, IGF-I and IGF-II stimulated P_4 production by granulosa cells with IGF-I being the more powerful promoter. It has been reported that INS effect on P_4 production may be via the IGF-I receptor. Thus, under normal circumstances it may not have physiological relevance to promoting P_4 production by granulosa cells (Spicer and Echternkamp, 1995). Concentrations of follicular fluid IGF-I and P_4 are correlated positively in postpartum anestrous cows, but are not significantly correlated in proestrus cows (Echternkamp et al., 1990; Samaras et al., 1993).

Circulating concentrations of P_4 are increased in cows supplemented with fat, which may have been due to a decrease in P_4 clearance rate and not to an increase in P_4 secretion by the corpus luteum. However, feeding 5.20 % fat did not affect follicular fluid concentrations of P_4, E_2, testosterone, cholesterol or TG from either large- or medium-size follicles (Lammoglia et al., 1997). On the other hand, cows with the most negative energy balance during first 9 wk of lactation secreted less P_4 in milk than cows in zero or slightly negative energy balance (Staples et al., 1990). Cows fed diets containing 1.8 % of DM as inert fat between wk 5 and 12 postpartum had similar concentrations of IGF-I but greater luteal phase secretion of P_4 than cows fed control diets (Spicer et al., 1993).

An important relationship has been established between energy balance and early reproductive response of the dairy cow. Thus, cows returning to estrus early after calving increased their food intake more rapidly and produced more milk than those cows that did not return to estrus (Lucy et al., 1992a). Anestrous cows with lower body weights failed to return to normal corpus luteum activity within 9 wk after calving, probably due to reduced DMI (Staples et al., 1990). Energy status of the cow is most important in determining return to estrous cycle activity after parturition. An increased loss of body weight during first 2 wk of lactation coincided with decreased ovarian activity of cows not returning to activity. Thus, the cows that failed to return to activity doubtless were in more negative energy balance due to fact that output of energy in milk was accelerating more rapidly than the increased energy from feed intake (Staples et al., 1990). The number of class 1 and 2 follicles before d 25 postpartum decreased with increasing energy

balance, whereas class 3 follicles increased (Lucy et al., 1991). Therefore, the key element dictating early ovulation in high producing dairy cows is the rapid increase in nutrient intake during the first few weeks after calving (Lucy et al., 1992a).

Other Metabolic Hormones

The protein hormone PRL from the anterior pituitary is structurally homologous to ST. Thus, PRL has growth promoting activity in some species and can stimulate IGF production. Additionally, PRL is well-known for its lactogenic and mammogenic actions and, in addition, has actions on the immune system, luteotropic actions, affects behavior, and control of testicular function (Sinha, 1995; Wallis, 1988).

Concentrations of PRL are significantly greater in bovine pre-colostrum and colostrum than in the maternal serum. These concentrations decline during the transition from production of colostrum to production of mature milk. Changes represent an accumulation of PRL in the highly concentrated prepartum secretions, but indicate relatively low secretion of PRL into milk during the postpartum period (Lee et al., 1995; Malven et al., 1987a). However, elevated concentrations of PRL during the periparturient period may not be related to subsequent rates of lactation because the basal concentrations may meet requirements for lactogenesis and lactation when the inhibitory effect of P_4 is removed at calving (Erb, 1976). Concentrations of PRL in prepartum mammary secretions were higher than plasma PRL concentrations in prepartum dairy cows (Pennington and Malven, 1985). Concentrations of PRL in plasma were in normal range when cows were fed diets containing fat and injected with bST (Lough et al., 1988).

Long daylight photoperiod and high ambient temperatures also increased plasma concentrations of PRL. Thus, high concentrations of PRL in circulation during periods of high ambient temperature were associated with increased secretion rate and reduced metabolic clearance rate (Igono et al., 1988).

Changes in the metabolism of glucose and INS in lactating cows treated with bST (33 mg/d for 28 d) suggested that treated cows had a greater ability to synthesize and conserve glucose than cows not injected during the same period (De Boer and Kennelly, 1989). However, glucose concentrations in plasma were not affected significantly by bST when they were injected from wk 9 to wk 20 of lactation, whereas glucose concentrations in plasma were higher at wk 20 in the group of cows fed high concentrate diet (Cisse et al., 1991). Treatment with ST did not change glucose utilization rate in 130 d old dairy calves (Head et al., 1970).

Thyroid hormones (T_3 and T_4) are essential for normal growth and development of many tissues. Thyroid hormones stimulate cell replication and cell differentiation; these growth effects may be related to direct actions or be mediated by ST, or by several growth factors, including the production of growth factors that regulate growth in autocrine fashion (Valcavi et al., 1992). Thyroid hormone plays a critical role in stimulation of pituitary ST synthesis and secretion. Thyroid hormone injections at physiological doses produce a rapid increase in pituitary ST content and subsequent increase in plasma ST concentration (Valcavi et al., 1992). Thyroid hormones also enhance action of IGF-I. Therefore, some of the growth promoting effects of thyroid hormone appears to be the result of their ability to interact with the ST-IGF-I axis. Thyroid hormone concentrations

were not affected by either level of concentrate fed or by bST injections (Bachman et al., 1992; Cisse et al., 1991).

From this brief description of a large and important array of hormones from different endocrine glands it can be seen that many factors can and do affect the secretion and concentrations of these important metabolic and reproductive hormones. Many of these hormones have a critical role during the transition period and during early lactation and are intimately associated with the ability of the cow to initiate and sustain a high level of milk production. Many hormones and growth factors also have a role in the ability of cows to ingest and partition nutrients needed to ensure lactation and favorable health status throughout the transition period and lactation. It was the objective of this research to evaluate and integrate some of the changes and actions of hormones and growth factor in cows during the transition period.

Materials and Methods

The second phase of this study was designed to evaluate plasma hormone, growth factor (IGF-I) and blood metabolite (glucose) concentrations of dairy cows during the transition period beginning d -21 before parturition and extending through d 65 postpartum. Blood samples from 46 dairy cows of the research herd at the DRU of the University of Florida were used. Distribution of experimental animals, management, feeding program and bST injections were described in detail in Chapter 3. Plasma harvested from blood samples collected throughout the experiment were frozen and stored at -20°C until assayed.

Glucose was assayed in all plasma samples by the automated procedure modified from that described by Gochman and Schmitz (1972). This colorimetric assay uses glucose oxidase to react with glucose to form hydrogen peroxide. This product reacts with a peroxidase indicator to form an intensely colored indamine dye to give a stable blue color that is read at 600nm. The amount of dye formed from the reaction is proportional to the amount of glucose in the plasma sample. All assays were completed using an automated analyzer (Bran + Luebbe, Model II) calibrated with standard curve of glucose concentrations from 0 to 100 mg/dl. Plasma samples were assayed directly without dilution after they had been centrifuged and filtered to remove small clots that could have affected the flow of sample through the tubing and membranes of the autoanalyzer. All samples from a cow were assayed on the same day.

Double antibody radioimmunoassay procedures were used to measure plasma concentrations of INS, PRL, ST, IGF-I, T_4, and T_3 during both the prepartum and postpartum periods. All samples were assayed in duplicate in a single assay. Concentrations of P_4 were measured by radioimmunoassay using reagents and antibodies described by Knickerbocker et al. (1986). All hormones, growth factor and metabolite were measured in all samples collected during the prepartum and postpartum periods; P_4 was measured only in postpartum samples.

Two second antibodies were prepared using Florida Native sheep housed and managed at the DRU. These antibodies were used in the radioimmunoassay of all hormones, except P_4. Rabbit gamma globulin or guinea pig gamma globulin were injected into sheep to prepare these antibodies. Either 20-25 mg of guinea pig gamma

globulin or 30-40 mg rabbit gamma globulin were placed in 25 ml Erlenmeyer flasks, 5 to 15 ml of distilled water were added and then stirred on a magnetic stirrer plate to solubilize the proteins. An equal amount of Freund's complete adjuvant was added and then mixed in micro-blender to the consistency of whipped cream (about 1 min on high speed). About 5 ml of this mixture was then injected subcutaneously (SC) into individual sheep over the shoulder on both left and right sides in three or four different sites. After 10-14 d, the same procedure was repeated except that the gamma globulins were mixed with Freund's incomplete adjuvant. After an additional 2 wk, sheep were bled from the jugular vein; 400 ml of blood were taken using a butterfly needle and 60 cc syringe to withdraw the blood. All blood was placed in 40 ml screw top centrifuge tubes, capped and placed on ice as soon as possible and then left overnight (16 h at 4 ^{o}C). Tubes containing the clotted blood were centrifuged at 5000 RPM for 30 min in an RC-3B centrifuge (Sorvall Instruments). Serum obtained was pooled and frozen. Sheep were bled again after an additional 4 wk. Sheep were reinjected with appropriate gamma globulin that was prepared with Freund's incomplete adjuvant by the procedure described at about 2-6 mo intervals. These pools of sheep anti-rabbit (SAR) and sheep anti-guinea pig (SAGP) sera were used in assays as the second antibody.

Insulin Assay

A double antibody radioimmunoassay procedure, as described by Soeldner and Sloane (1965) and modified by Malven et al. (1987b) was used for assay of INS in plasma samples. Highly purified INS (Sigma Immunochemicals, St. Louis, MO) was weighed

(≈100μg) as Stock INS and dissolved in 30 mM HCL (pH=2.5) using an ultrasonic water bath. The INS then was aliquoted (10 μl) into 1 ml microfuge vials and frozen to be used for the iodination procedure. The Stock INS was diluted in assay buffer (borate/BSA; 0.133 M borate, 0.01% merthiolate, with 0.5% BSA) to give a final concentration of 100 ng/ml for preparation of standards which contained 0.3, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 15, 20, 25, and 30 ng INS/ml. The first antibody (primary), guinea pig anti-bovine INS, was obtained from Sigma Chemical Co, St. Louis, MO. It was dissolved in borate/BSA buffer (1:20,000). The second antibody, SAGP, was prepared as described and used at 1:3-1:5 dilutions in borate/EDTA assay buffer.

A constant volume of plasma (100 or 150 μl) was used for assay. Greater or lesser volumes of sample were used if the resulting hormone concentration in the sample was very low or very high. Samples were assayed in duplicate along with 100-150 μl assay buffer in 12 x 75 borosilicate tubes. Immediately thereafter, 100 μl iodinated INS and 100 μl diluted primary antiserum were added to all tubes. After incubation for 24 h at 4^0C, diluted second antibody (100 μl), SAGP, and 100 μl of normal guinea pig serum (NGPS) were added to all tubes except the total count tubes, and incubated for 10 min. After that, 750 μl of 15% polyethylene glycol (PEG) in borate buffer were added to all tubes except the total count tubes, and tubes were vortexed and after 10 min the tubes were centrifuged at 3000 RPM for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain and dry. After tubes were dry, bound radioactivity in tubes was measured using a Packard^R auto gamma counter (model B-5005). Final results were calculated using the spline radioimmunoassay data processing procedure.

For iodination and separation of INS one vial containing 10 µl INS (0.5 µg/µl) was thawed 1 h prior to iodination, then 10 µl of 0.5 M phosphate buffer were added. The column used to separate the iodinated protein from free iodine was prepared by cutting off the mouthpiece of a 10 ml disposable glass pipette. A small glass bead or glass wool plug was placed into the column before adding the Sephadex packing (Sephadex G-50, Sigma Chemical; dispersed in 0.01 M phosphate buffer) until the Sephadex filled the column. Subsequently, the column was washed with 2 ml of 0.5 M phosphate buffer that contained 0.5 % BSA followed by 10 ml 0.01 M phosphate buffer.

Phosphate buffer (0.01 M) was retained at the top of the Sephadex bed until it was used for protein separation. Immediately prior to the iodination, 3 mg of chloramine-T and 5 mg sodium metabisulfite were weighed then each was dissolved in 1 ml 0.5 M

Table 4-1. Arrangement of assay tubes for determination of INS.

Tubes[a]	Assay Buffer[b]	Sample	1st Ab	I^{125}INS	Plasma
TCT	-------	-------	-------	100	------
NSB	400	-------	-------	100	-------
NSB-P	300	-------	-------	100	100
Zero	300	-------	100	100	-------
Standards	200	100	100	100	-------
Samples	200	100	100	100	-------

[a]TCT=total radioactivity count tube, NSB=non-specific binding for buffer, NSB-P=non-specific binding for plasma, Zero=reference, no cold INS added. Volumes are in µl.

phosphate buffer. Sequentially, 0.5 M phosphate buffer (20 µl), radioactive iodine (I^{125}: 1 mCi, 10 µl) and 10 µl chloramine-T were added to the vial that contained the INS. The reaction lasted 15-45 sec, during which contents were mixed by finger tapping; then 10 µl

sodium metabisulfite and 30 µl 0.01 M phosphate buffer were added to the vial. This solution was mixed and transferred to the top of the Sephadex column; then the reaction vial was rinsed with 50 µl 0.01 M phosphate buffer to ensure complete transfer of the iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1-40 were filled with 500 µl 0.5 M borate-BSA buffer. Tubes were placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 µl) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity in a Tracor Analytic Gamma Counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak was free I^{125}. The iodinated INS was stored at 4 ^{0}C and used within 10 d of iodination.

Prolactin Assay

A double antibody radioimmunoassay procedure was used, as described by Malven and McMurtry (1973), and modified by Chew et al. (1976). Highly purified PRL supplied by USDA was dissolved (0.5 μg/μl) in 0.01 M $NaHCO_3$ (pH=8.0) to make Stock 1 which contained 55,000 ng/ml. This was aliquoted (10 μl) in 1 ml microfuge vials and frozen to be used for the iodination procedure. Stock 2 was made by adding 91 μl of Stock 1 and making it up to 50 ml with phosphate/BSA buffer (0.01 M phosphate/1.0 % BSA), for preparation of standards which contained 0.05,0.1, 0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 2.5, and 3.0 ng PRL/100 μl. The first antibody (primary), rabbit anti-bovine

PRL, also was obtained from USDA. It was diluted (1:120,000) in assay buffer which contained 1.0 % BSA (PO_4 /BSA). The second antibody, SAR, was prepared as described and diluted 1:3 in EDTA-PBS for use.

Immediately thereafter, 100 μl iodinated PRL and 100 μl diluted primary antiserum were added to all tubes, except total count and non-specific binding tubes. After incubation for 20-30 h at 4 °C, diluted second antibody (100 μl) SAR and 100 μl normal rabbit serum (NRS) were added to all tubes except the total count tubes. Then, 1.5 ml of 6% PEG were added to all tubes and they were vortexed. After that tubes were centrifuged at 3000 RPM for 30 min at 4°C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain. After tubes were dry, bound radioactivity in the tubes was measured using a Packard^R auto gamma counter (model B-5005). Final results were calculated using the spline radioimmunoassay data processing procedure. Plasma samples were assayed after dilution with buffer. Greater or lesser quantities of sample were assayed if the resulting hormone concentration in the sample was very low or very high.

For iodination and separation of PRL one vial containing 10 µl PRL (0.5 µg/µl) was thawed 1 h prior to iodination and then 10 µl 0.01 M phosphate buffer was added to the thawed aliquot. The column used to separate the iodinated protein from free iodine was prepared by cutting off the mouthpiece of a 10 ml disposable glass pipette. A small glass bead or glass wool plug was placed into the column before adding the Sephadex column packing (Sephadex G-75, Sigma Chemical; dispersed in 0.01 M phosphate buffer) until the Sephadex filled the column. Subsequently, the column was washed with

2 ml 0.5 M phosphate buffer that contained 1.0% BSA, followed by 10 ml 0.01 M phosphate buffer. Some buffer was retained in the column at the top of Sephadex bed until it was used for protein separation.

Immediately prior to the iodination, chloramine-T (3 mg) and sodium metabisulfite (5 mg) each were dissolved in 1 ml phosphate buffer. Sequentially, 0.5 M phosphate buffer (20 μl), radioactive iodine (I^{125} ; 10 μl) and 10 μl chloramine-T were added to the vial that contained the PRL. After a reaction time of 20-25 sec, during which contents were mixed by finger tapping the vial, 10 μl sodium metabisulfite and 30 μl of 0.01 M phosphate buffer were added to the vial. This solution was transferred to the top of the Sephadex column; then the reaction vial was rinsed with 50 μl 0.01 M phosphate buffer to ensure complete transfer of iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1-40 were filled with 500 μl 0.5 M phosphate-BSA buffer. Tubes were placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 μl) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity with a Tracor Analytic Gamma Counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak represented free I^{125}. The iodinated PRL was stored at 4 ^{0}C and used within 10 d of iodination.

Table 4-2. Arrangement of assay tubes for determination of PRL.

Tubes	Assay Buffer[2]	Sample	1st Ab	I^{125} PRL	Plasma
TCT	-------	-------	-------	100	-------
NSB	400	-------	-------	100	-------
NSB-P	390	-------	-------	100	10
Zero	300	-------	100	100	-------
Standards	200	100	100	100	-------
Samples	290	10	100	100	-------

[1]TCT=total radioactivity count tube, NSB=non-specific binding for buffer, NSB-P=non-specific binding for plasma, Zero=reference, no cold PRL added;
[2]Volumes are in µl.

Insulin-Like Growth Factor-I (IGF-I) Extraction and Assay

A double antibody radioimmunoassay procedure, as described by Abribat et al. (1990) and modified for sample extraction by Enright et al. (1989) and Daughaday et al. (1980), was used for IGF-I determinations.

For the extraction of IGF-I from its binding proteins, the method of Enright et al. (1989) was used for plasma samples. A mixture of ethanol : acetone : acetic acid (EAA 60 : 30 : 10, by volume) was used for extraction. Exactly 400 μl of extraction mixture were added to 100μl of plasma sample in 12 x 75 mm polystyrene tubes. The mixture was mixed thoroughly for 15 sec on a vortex and allowed to stand for 30 min after mixing. Tubes then were centrifuged at 3000 RPM for 30 min in a refrigerated centrifuge (RC-2B). Exactly 250 μl of the supernatant were transferred to 12 x 75 mm polystyrene tubes; then 100 μl of 0.855 M Trizma base were added to neutralize the extraction mixture. After that 350 μl of the assay buffer were added to make the final dilution 1:14.

Highly purified Human IGF-I, supplied by Upstate Biotechnology Incorporated (Richmond, CA), was dissolved (0.5 μg/μl) in 0.1 M acetic acid (pH=2-5), and then 10 μl aliquots were transferred to 1 ml microfuge vials and frozen to be used for the iodination procedure.

Highly purified Bovine IGF-I; supplied by Monsanto Company (St. Louis, MO) was dissolved (10 μg in 100 μl of 0.1 M acetic acid) to give Stock 0 (100 ng/ml), and then this was aliquoted into tubes (10 μl/ tube) and frozen. To make Stock 1, 10 μl of Stock 0 was added to 490 μl of assay buffer. Stock 2 was made by adding 10 μl of Stock 1 to 990 μl of assay buffer; this had a final concentration of 20 pg/ml. Using Stock 2, standards were prepared to contain 50, 100, 200, 300, 400, 500, 600, 800, 1,000, 1,200, 1,500 and 2,000 pg IGF-I/ml. The first antibody (primary), rabbit anti-bovine IGF-I, was obtained as a gift from Drs. Louis Underwood and Judson J. Van Wyk, Division of Pediatric Endocrinology, University of North Carolina, Chapel Hill, NC. It was dissolved (1:4,000) in assay buffer (200 mg/L protamine, 4.4 g/l sodium monobasic phosphate, 10 ml of 2% of sodium azide, 3.72 g/l EDTA (0.013 M) and 2.5 g/l BSA). The second antibody (SAR) was prepared and used at 1:3-1:5 dilutions in EDTA-PBS.

Plasma sample extracts were used in the assay (10-20 μl of plasma extract and 190 μl assay buffer). Greater or lesser quantities of sample extract were used if the resulting growth factor concentration in the sample was very low or very high. Immediately thereafter, 100 μl iodinated IGF-I and 100 μl diluted primary antiserum were added to all tubes, except total count and non-specific binding tubes. After incubation for 20-30 h at 4^0C, diluted second antibody (100 μl), SAR, and 50 μl NRS (1:50) were added

to all tubes except the total count tubes and allowed to stand 30 min. After that, 1 ml of 6% PEG in assay buffer was added, vortexed and allowed to stand for 15 min; then tubes were centrifuged at 3000 RPM for 30 min at 4 °C (RC-3B, refrigerated centrifuge, Sorvall Instruments). Then they were decanted and inverted on absorbent paper to drain. After tubes were swabbed dry, or allowed to dry overnight, bound radioactivity in tubes was measured using a Packard[R] auto gamma counter (model B-5005). Final results were calculated using the spline radioimmunoassay data processing procedure.

Table 4-3. Arrangement of assay tubes for determination of IGF-I.

Tubes[1]	Assay Buffer[2]	Sample	1st Ab	I^{125} IGF-I	Plasma Extract
TCT	-------	-------	-------	100	-------
NSB	300	-------	-------	100	-------
NSB-P	290	-------	-------	100	10
Zero	200	-------	100	100	-------
Standards	100	100	100	100	-------
Samples	190	10	100	100	-------

[1]TCT=total radioactivity count tube, NSB=non-specific binding for buffer, NSB-P=non-specific binding for plasma, Zero=reference, no cold IGF-I added;
[2]Volumes are in µl.

For iodination and separation of IGF-I one vial containing 10 µl IGF-I (0.1 µg/µl) was thawed 1 h prior to iodination; then 10 µl 0.01 M phosphate buffer were added to the thawed aliquot. The column used to separate the iodinated protein from free iodine was prepared by cutting off the mouthpiece of a 10 ml disposable glass pipette. A small glass bead or glass wool plug was placed into the column before adding the Sephadex column packing (Sephadex G-50, Sigma Chemical; dispersed in 0.01 M phosphate buffer) until

the Sephadex filled the column. Subsequently, the column was washed with 2 ml 0.5 M phosphate buffer that contained 1.0% BSA followed by 10 ml 0.01 M phosphate buffer. Buffer (0.01 M) was retained at the top of the column to cover Sephadex packing until used for protein separation.

Immediately prior to the iodination, chloramine-T (3 mg) and sodium metabisulfite (5 mg) each were dissolved in 1 ml phosphate buffer. Sequentially, 0.5 M phosphate buffer (20 μl), radioactive iodine (I^{125} ;5 μl 0.5 mci.) and 10 μl chloramine-T were added with mixing to the vial that contained IGF-I. After a reaction time of 25 sec, with mixing, 10 μl sodium metabisulfite and 30 μl 0.01 M phosphate buffer were added to the vial and mixed. This solution was transferred to the top of the Sephadex column; then the reaction vial was rinsed with 50 μl 0.01 M phosphate buffer to ensure complete transfer of iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1-50 were filled with 500 μl 0.5 M phosphate-BSA buffer and placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 μl) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity using a Tracor Analytic Gamma counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak represented free I^{125}. The iodinated IGF-I was stored at 4 ^{o}C and used within 10 d of iodination.

Somatotropin Assay

A double antibody radioimmunoassay procedure, as described by Malven and McMurtry (1973), was used for assay of ST in plasma samples. Purified ST, provided by the USDA reproduction laboratory (Beltsville,MD), was weighed (350μg) as Stock ST and dissolved in 350μl 0.01 M $NaHCO_3$ (pH=9.5) using an ultrasonic water bath. The ST then was aliquoted (20 μl) into 1 ml microfuge vials to be used for iodination. The Stock ST was diluted in assay buffer (0.01 M PO_4/ 1% BSA) to give a final concentration of 100 ng/ml for preparation of standards which contained 1.0, 2.0, 4.0, 6.0, 8.0, 10, 15, 20, 25, 30, 40, and 50 ng ST/ml. The primary antibody, rabbit anti-bovine ST, was obtained from USDA (Beltsville, MD). It was dissolved in 0.01 M PO_4/ buffer (1:40,000). The second antibody, SAR, was prepared as described previously and diluted 1:3-1:5 in EDTA-PBS. Normal rabbit serum was diluted 1:100 with 0.01 M PO_4/BSA for use in the assay.

Aliquots of standards (100 μl) and plasma samples (100 μl) and 200 μl 0.01 M PO_4/BSA were added to assay tubes; total assay volume was 500 μl. The arrangement and volumes of all individual assay tubes is in table 4-4. Standards and samples were assayed in triplicate and duplicate, respectively. Immediately after pipetting samples and buffer into assay tubes, 100 μl diluted primary antiserum was added to all tubes except total count and non-specific binding tubes. After pipetting, all assay tubes were incubated for 18-24 h at 4°C. Following incubation, iodinated ST (100 μl) was added to all assay tubes and incubated again for 24 h at room temperature. Diluted SAR(100 μl) and 100 μl of NRS then were added to all tubes except the total count tubes, and incubated for 10

min. Next, 750 μl of PO_4 buffer with 15% polyethylene glycol (PEG) in assay buffer were added to all tubes except the total count tubes, and tubes then were vortexed. Tubes then were centrifuged at 3000 RPM for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain. After tubes had been swabbed dry, bound radioactivity in tubes was measured using a Packard^R auto gamma counter (model B-5005). Results were calculated using the spline curve fit procedure for radioimmunoassay data processing.

For iodination and separation of ST the column used was prepared by cutting off the mouthpiece of a 10 ml disposable glass pipette. A small glass bead or glass wool plug was placed into the bottom of the column before adding the Sephadex packing (Sephadex G-75, Sigma Chemical; dispersed in 0.01 M phosphate buffer) until the Sephadex filled the column (15 cm). Subsequently, the column was washed with 2 ml 0.5 M phosphate buffer that contained 0.5 % BSA followed by 10 ml 0.01 M phosphate buffer. Phosphate buffer (0.01 M) was retained at the top of the column until it was used for protein separation.

Somatotropin was iodinated using IODO-GEN (Pierce, Rockford, IL) as the reaction catalyst. IODO-GEN was dissolved in chloroform to obtain a 0.1 mg/ml Stock solution. Duplicate 12x75 mm borosilicate tubes were washed with glacial acetic acid, rinsed with deionized water, and dried for use as iodination reaction tubes. IODO-GEN Stock solution (20 µl) then was added to the reaction tube to give a total concentration of 2 µg and air dried to coat the reaction tube. Sequentially, radioactive iodine (I^{125}: 1 mCi, 10 µl), solubilized ST (20 µl), and 0.01 M phosphate buffer (20 µl) were added to the reaction tube. The reaction lasted 4-8 min, during which contents were mixed by finger

tapping. This solution was transferred to the top of the Sephadex column; then the reaction vial was rinsed with 50 µl 0.01 M phosphate buffer to ensure complete transfer of the iodinated protein.

Borosilicate tubes (15 x 100 mm) numbered 1 to 35 were filled with 500 µl 0.5 M phosphate-BSA buffer. Tubes were placed in a fraction collector to receive eluate (20 drops) from the Sephadex column. Aliquots (10 µl) of each eluted fraction were transferred to a second set of tubes for use in identifying elution peaks by counting radioactivity in a Tracor Analytic Gamma Counter (Nuclear-Chicago, Gamma Trac 1191, G. D. Searle and Co., Des Plaines, IL). Collection tubes that contained the first large peak, which corresponded to I^{125} bound to protein, were pooled. The second large peak was free I^{125}. The iodinated ST was stored at 4 ^{0}C and used within 10 d of iodination.

Table 4-4. Arrangement of assay tubes for radioimmunoassay of ST.

Tube[1]	Buffer	Sample	1st Ab	I^{125}-ST	Plasma
TCT	---	---	---	100	---
NSB	400	---	---	100	---
NSB-P	300	---	---	100	100
Zero	300	---	100	100	---
Standard	200	100	100	100	---
Sample	200	100	100	100	---

[1]All volumes are in µl. TCT represents total count tube. NSB represents buffer non-specific binding. NSB-P represents plasma non-specific binding. Zero is reference, no cold ST added.

Triiodothyronine Assay

A double antibody radioimmunoassay procedure was used for assay of T_3. Highly purified L-tri-iodothyronine free acid (Sigma Chemical Co., St. Louis, MO), was dissolved in 0.05 M NaOH to make Stock 1 solution which contained 1.0 mg/ml. The Stock 1 solution was diluted again in 0.05 M NaOH to 25 μg/ml for use as Stock 2 solution. Stock 2 then was diluted in stripped plasma to obtain a concentration of 100 ng/ml for use in standard preparation. Normal bovine plasma was stripped of thyroid hormones using activated charcoal (Fisher Scientific, Springfield, NJ) for dilution of Stock solution 2. Standards were prepared which contained 0.1, 0.2, 0.4, 0.6, 1.0, 2.0, 3.0, 4.0, and 6.0 ng T_3/ml. The Primary antibody, rabbit anti-bovine T_3 (ICN immunobiologicals, Lisle, IL) was diluted (1:3,000) in assay buffer which contained 1.0% BSA (0.01 M PO_4/BSA). The second antibody, SAR, was prepared for use as described previously and was diluted 1:5 in 0.05 M EDTA-PBS. Normal rabbit serum (NRS) was diluted 1:100 with PO_4/BSA for use in the assay.

Aliquots of T_3 standards (100 μl) and plasma samples (100 μl) were prepared in 250 μl 0.01 M PO_4/BSA. Standards and samples were assayed in triplicate and duplicate, respectively. Immediately thereafter, 100 μl iodinated T_3 (NEN Research Products, Du Pont Co. Boston, MA) and 100 μl diluted primary antibody were added to all tubes except total count and non-specific binding tubes. Arrangement and volumes for specific assay tubes are in table 4-5.

After incubation for 24 h at room temperature, diluted SAR (100 μl) and 100 μl NRS were added to all tubes except the total count tubes. Then 750 μl of 6% PEG were

added and all tubes, except total count tubes, were vortexed, and then were centrifuged at 3000 RPM for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain. After tubes had been swabbed dry, bound radioactivity in the tubes was measured using a Packard^R auto gamma counter (model B-5005). Results were calculated using the spline radioimmunoassay procedure for data processing.

Table 4-5. Arrangement of assay tubes for radioimmunoassay of T_3.

Tube[1]	Buffer	Sample	1st Ab	T_3-I^{125}	Plasma[3]
TCT	---	---	---	100	---
NSB	350	---	---	100	100
Zero	350	---	100	100	---
Standard	250	100	100	100	---
Sample	250	100	100	100	---

[1]All volumes are in µl. TCT represents total count tube. NSB represents non-specific binding. [2]Stripped plasma. Zero is reference, no cold T_3 added.

Thyroxine Assay

A double antibody radioimmunoassay procedure, similar to that described for T_3, was used to assy samples of plasma for T_4. Highly purified L-thyroxine free acid (2 mg; Sigma Chemical Co., St. Louis, MO) was dissolved in 0.05 M NaOH to make Stock 1 solution which contained 1.0 mg/ml. The Stock 1 solution was diluted in 0.05 M NaOH to 25 µg/ml for use as Stock 2 solution. Stock 2 then was diluted in stripped plasma to a concentration of 100 ng/ml for use in standard preparation. Normal bovine plasma was stripped of T_4 using activated charcoal (Fisher Scientific, Springfield, NJ) for dilution of

Stock solution 2. Standards were prepared that contained 2.0, 4.0, 6.0, 8.0, 16, 30, 60, 100, 200 and 360 ng T_4/ml. The Primary antibody, rabbit anti-bovine T_4 (ICN immunobiologicals, Lisle, IL) was diluted (1:7,000) in assay buffer which contained 1.0% BSA (0.01 M PO_4/BSA). The second antibody, SAR, was prepared as described previously and diluted 1:5 in 0.05 M EDTA-PBS. Normal rabbit serum (NRS) was diluted 1:100 with PO_4/BSA buffer for use in the assay.

Aliquots of standards (25 μl) and plasma samples (25 μl) were added to 325 μl 0.01 M PO_4/BSA. Standards and samples were assayed in triplicate and duplicate, respectively. Immediately thereafter, 100 μl iodinated T_4 (NEN Research Products, Du Pont Co. Boston, MA) and 100 μl diluted primary antibody were added to all tubes, except total count and non-specific binding tubes. Arrangement and volumes for specific assay tubes are in table 4-6.

After incubation for 24 h at room temperature, diluted SAR (100 μl) and 100 μl NRS were added to all tubes except the total count tubes. Then, 750 μl of 6% PEG were added and all tubes, except total count tubes, and they were vortexed. After that tubes were centrifuged at 3000 RPM for 30 min at 4 ^{0}C (RC-3B, refrigerated centrifuge, Sorvall Instruments), decanted, and inverted on absorbent paper to drain. After tubes were swabbed dry, bound radioactivity in the tubes was measured using a Packard^R auto gamma counter (model B-5005). Results were calculated using the spline radioimmunoassay procedure for data processing.

Table 4-6. Arrangement of assay tubes for radioimmunoassay of T_4.

Tube[1]	Buffer	Sample	1st Ab	T_4-I^{125}	Plasma[2]
TCT	---	---	---	100	---
NSB	325	---	---	100	25
Zero	350	---	100	100	---
Standard	325	25	100	100	---
Sample	325	25	100	100	---

[1]All volumes are in µl. TCT represents the total count tube. NSB represents non-specific binding. [2]Stripped plasma. Zero is reference, no cold T_4 added.

Statistical Analyses

As described for data collected and analyzed in chapter 3, data collected from laboratory analyses of plasma samples were analyzed as a sequential experiment in two sections. First section included data for samples collected during the 3 wk prepartum. Data were analyzed as completely randomized design (CRD). Data were analyzed by least squares analysis of variance procedures of SAS (1991). Analyses performed included those for ST, INS, PRL, T_4, T_3, IGF-I, HCT, PP and glucose. Data were analyzed for the overall prepartum period and also for specific days within the prepartum period (-14, -7, -3, -1 and 0). Models included the main effect of prepartum treatment (PRTRT), cow (PRTRT) and cubic polynomial of days for the overall prepartum period.

Data recorded from laboratory analyses of plasma collected after parturition constituted the second section. These data were analyzed as a 2x2x2 factorial arrangement, as there were two prepartum treatments (groups I and II), two diets (0 and 15 % WCS) and two bST treatments after calving (injected or not injected with 5 mg

bST/d). Analyses performed included those for ST, INS, PRL, T_4, T_3, IGF-I, HCT, PP, P_4 and glucose. Data were analyzed in two ways, first for the transition period from d -21 before parturition to d +21 after parturition, and second, as an overall postpartum period from d 0 to 65 d postpartum. Models included the main effects of prepartum treatment (PRTRT), diet, postpartum treatment (PPTRT) the two-factor interactions of PRTRT*diet, PRTRT * PPTRT, and PPTRT*diet, the three-factor interaction of PRTRT*diet*PPTRT, and the effect of cubic order polynomial of days.

Regression analyses were performed to describe the effect of treatment for ST, INS and IGF-I during the transition period and for P_4 during the overall postpartum period. Tests for heterogeneity of regression then were performed to determine whether regression curves were parallel.

Results and Discussion

Objectives of this study were to evaluate changes in plasma concentrations of metabolic hormones, the growth factor IGF-I, glucose and blood parameters during the transition period from -21 d before calving through +21 d postpartum, and plasma concentrations of P_4, as an indicator of ovarian activity during the early lactation period up to d 65. Recorded data during the prepartum period included plasma concentrations of ST, INS, PRL, T_4, T_3, IGF-I, glucose, PP, and HCT. Twenty four Holstein cows were randomly assigned to each of the two bST treatment groups before parturition, as described in chapter 3.

No differences between bST treatment groups were detected for mean concentrations of PRL, T_4, T_3, INS, IGF-I, HMT or PP during the overall prepartum period from d -21 to d 0 (calving day). In contrast, treatment differences were detected for plasma concentrations of ST and glucose during this period. Least squares analyses of variance are in table 4-7. Results showed significant effects of PRETRT for both ST ($P<0.0024$) and glucose ($P < 0.0294$) but not for IGF-I. A significant linear effect of days was observed for glucose, INS, and IGF-I.

Least squares means and SE for dependent variables during the over all prepartum period and for specific days (-14, -7, -3, -1 and 0) are in table 4-8. Mean concentrations of ST during the overall prepartum period were higher for the bST-treated group of cows, as expected (6.83 vs. 3.59 ng/ml). Additionally, results for specific days indicated that concentration of ST for bST-treated cows were greater than for cows not treated throughout the prepartum period. Interestingly, both groups of cows showed a progressive increase in plasma concentrations of ST from d -14 to d 0. However, on calving day (d 0) plasma concentrations of ST for bST-treated cows were nearly double those on d -1 (d -1= 7.63; d 0 = 13.52 ng/ml), whereas for not treated cows ST concentrations for these 2 d did not show a similar increase (d -1 = 6.35; d 0 = 7.47 ng/ml). These results agreed with previous findings that plasma concentrations of ST increased and the increase was maintained higher (6.5 and 22.7 ng/ml) when cows were injected with 5 or 14 mg bST/d (Simmons et al., 1994) or 25 mg bST/d (Bachman et al., 1992) during the prepartum period; uninjected cows had a concentration of 1.6 ng/ml on the same day. Cows injected with bST during the lactation showed a similar sustained

Table 4-7. Least Squares Analyses of Variance for ST, INS, Glucose, and IGF-I of Holstein Cows During Prepartum Transition Period (-21 d to calving).

	ST			INS			Glucose			IGF-I		
Source	df	MS	Pr>F	df	MS	Pr>F	df	MS	Pr>F	df	MS	Pr>F
Pretrt[1]	1	517.26	0.0024	1	0.136	0.3620	1	1016.02	0.0294	1	6286.56	0.2508
Cow (Pretrt)	44	49.72	0.0333	44	0.160	0.0001	44	200.44	0.0001	44	4641.49	0.0001
Days	1	0.49	0.9027	1	0.147	0.0077	1	107.77	0.0395	1	62268.04	0.0001
Error	153	32.71		153	0.020		153	25.00		151	801.49	

[1]Prepartum treatment.

Table 4-8. Least Squares Means and SE for Hormones and Growth Factor Concentrations and Blood Measures of Holstein Cows for Specific Days for Treatment I During the Prepartum Transition Period[1].

Days	Overall		-14		-7		-3		-1		0	
Measures	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
ST (ng/ml)	3.59	0.59	2.72	0.74	2.52	0.91	3.78	1.08	6.35	1.94	7.47	2.97
INS (ng/ml)	0.57	0.01	0.65	0.09	0.68	0.26	0.52	0.08	0.39	0.07	0.32	0.03
IGF-I (ng/ml)	125.65	2.95	145.39	12.35	152.38	55.60	106.29	13.79	84.55	25.11	55.02	15.57
PRL (ng/ml)	10.15	0.91	9.20	2.50	7.75	2.29	7.67	1.57	45.12	15.22	57.78	9.38
T_4 (ng/ml)	80.94	1.77	99.06	14.28	86.44	32.53	59.04	7.98	28.13	75.77	75.77	29.90
T_3 (pg/ml)	611.20	12.07	605.83	87.02	487.10	268.70	610.34	109.25	326.27	110.49	310.17	137.75
P_4 (ng/ml)											18.28	5.91
HMT%	30.18	0.20	29.30	0.84	31.33	0.88	30.13	0.91	28.17	3.75	32.05	1.16
PP%	6.19	0.05	6.13	0.14	6.15	1.05	6.03	0.21	6.17	0.60	5.48	0.25
Glucose (mg/dl)	62.01	0.51	64.96	2.53	68.33	0.81	59.94	3.64	63.97	12.14	70.63	6.60

[1]Cows not injected with bST during prepartum period.

Table 4-8 -- Continued

Days	Overall		-14		-7		-3		-1		0	
Measures	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
ST (ng/ml)	6.83	0.56	5.14	4.88	4.84	1.22	4.63	0.74	5.63	0.88	7.47	2.97
INS (ng/ml)	0.63	0.01	0.53	0.21	0.68	0.07	0.75	0.22	0.44	0.08	0.32	0.03
IGF-I (ng/ml)	137.02	2.79	119.11	37.61	140.92	10.67	109.87	18.40	56.09	9.09	55.02	15.57
PRL (ng/ml)	12.64	0.86	21.29	3.74	8.60	2.29	10.06	2.44	35.74	9.05	57.78	9.38
T_4 (ng/ml)	80.58	1.69	56.04	33.95	91.57	9.20	61.95	6.46	44.05	4.15	75.77	29.90
T_3 (pg/ml)	634.55	15.92	275.10	220.15	667.88	73.43	828.86	177.81	540.76	89.58	310.17	137.75
P_4 (ng/ml)											18.28	5.91
HMT%	30.45	0.19	28.25	0.35	30.35	0.71	29.75	1.72	30.55	0.52	32.05	1.16
PP%	6.44	0.04	6.20	0.94	6.58	0.29	6.27	0.40	6.59	0.28	5.48	0.25
Glucose (mg/dl)	66.55	0.49	60.33	4.79	70.53	1.32	67.15	2.41	65.66	3.11	70.63	6.60

[1]Cows inject with 5 mg bst/d during prepartum period.

increase in plasma ST concentrations (Lucy et al., 1993). Somatotropin is a homeorhetic hormone that is involved in the regulation of the metabolic adaptations that occur during the transition from the nonlactating to lactating state. Consequently, ST concentrations start to increase during late pregnancy and reach highest peak concentrations at calving and then they decline during the postpartum period to moderately high levels that are sustained throughout early lactation. Thus, ST exerts a powerful galactopoietic influence once lactation is established. And it alters tissue response to INS and cathecolamines (Bauman and Vernon, 1993). Therefore, ST decreases rates of lipogenesis and activities of lipogenic enzymes and promotes glucose utilization for milk synthesis.

Although mean concentrations of INS did not differ for the bST-treated and not treated cows during the prepartum period (table 4-8), plasma concentrations of INS tended to decrease from d -21 to d 0 but there were no great differences in the decrease across treatments groups. This agreed with previous reports (Bachman et al., 1992; Gallo and Block, 1990b). However, there are reports that INS concentrations were increased by bST injections during late lactation and the dry period but not during the subsequent lactation. These changes have been associated with greater amounts of circulating glucose during late lactation and dry period because of the positive energy balance of the cows during these periods (Soderholm et al., 1988; Vicini et al., 1991). Conversely, INS concentrations were decreased to about 50% of previous concentrations when cows were fed supplemental fat and injected with bST; these treatments were associated with reduced DMI that occurred due to fat in the diet (Cisse et al., 1991; Lough et al., 1988).

Results presented in table 4-8 also describe plasma concentrations of IGF-I during the overall prepartum period. Again, no differences were observed between treatment groups. Cows in both groups showed high plasma concentrations of IGF-I on d -14; concentrations were 145.9 and 140.2 ng/ml for not treated and treated cows. Plasma concentrations of IGF-I decreased progressively as cows approached parturition; and on d 0 concentrations were only about one-third those 2 wk earlier (55.02 and 44.43 ng/ml for not treated and treated cows). Plasma concentrations of IGF-I and II are under partial control of ST. Nutritional status likely plays a major role in the regulation of the somatotropic-axis (Breier et al., 1986; Maes et al., 1983b; Phillips and Unterman, 1984) and in this way affects concentrations of ST and IGF-I. Results from the current study differed from those reported by Simmons et al. (1994). They found that concentrations of IGF-I increased concomitant with injections of 5 or 14 mg bST/d or 25 mg bST/d as reported by Bachman et al. (1992). However, Simmons et al. (1994) also reported that ST concentrations remained elevated, whereas plasma concentrations of IGF-I decreased from d -21 to d -1; and thus results of current study for IGF-I were comparable to those reported by Simmons et al. (1994) and Vicini et al. (1991). The uncoupling of the ST and IGF-I responses observed may have been due to the decreased DMI of bST-treated cows during the week before calving. Reduced DMI of growing steers caused a decrease in basal concentrations of IGF-I and abolished the response of IGF-I and II to exogenous bST (Breier et al., 1988; Ronge and Blum, 1989). The reason for this uncoupling may be that increased concentrations of ST, due to secretion or injection of bST, shifts proportions of IGFBPs in plasma and changes in the binding of the IGFs to these proteins

(Armstrong and Benoit, 1996). Of course, there are many associated changes in the physiology of the cow as parturition approaches and one or more of these changes could have been a contributor to the changes observed in ST and/or IGF-I or in the uncoupling of the axis.

During early lactation, when low nutrient intakes occur, the circulating concentrations of IGF-I decrease (Ronge et al., 1988; Spicer et al., 1990; Vicini et al., 1991). During the dry period, when cows were in positive energy balance, bST significantly increased ($P < 0.08$) plasma concentrations of IGF-I (Bachman et al., 1992). A lesser need for IGF-I once dry period mammary growth has been completed and the accumulation of IGFs in pre-colostrum and colostrum has ended may reduce secretion of IGF-I and coordinated changes in its secretion and plasma concentrations occur, perhaps due, in part, to reduced ST concentrations in plasma.

Interestingly, plasma glucose concentrations of bST treated cows during the prepartum period were about 7.3% greater than for cows not treated (66.56 vs. 62.01 mg/dl). It has been suggested that bST treatment may affect plasma concentrations of glucose indirectly via its INS-antagonizing activity and/or directly by promoting gluconeogenesis. It has been reported that hepatic glucose production from propionate is enhanced during bST treatment (McDowell, 1991) and ST decreased the ability of INS to inhibit gluconeogenesis (Sechen et al., 1990). Reduced INS concentrations and, therefore its subsequent actions in lactating cows, would favor greater glucose availability for mammary uptake and use in the synthesis of milk. These actions of injected bST on INS certainly would favor the increased synthesis of milk. So while the increase in glucose

concentrations observed were not large they could result in greater availability of glucose to support lactation. Clearly, glucose is a key substrate for milk synthesis and is the first limiting energy substrate for the lactating mammary gland (Davis and Collier, 1985).

Because the objectives of this study were to evaluate metabolic response of cows injected with bST during the transition period from d -21 prepartum to d +21 postpartum, a second series of analyses was performed that considered this time period. Analyses of variance for the metabolic hormones and blood parameters detected significant effects of PRETRT ($P < 0.601$) and PPTRT ($P < 0.0214$) on ST. Least squares means for ST concentrations due to prepartum treatment (PRETRT) with bST were 7.72 vs. 5.88 ng/ml and 7.93 vs. 5.70 ng/ml for postpartum treated and not treated cows. No significant effects of bST treatment prepartum or postpartum were detected on any of the dependent variables evaluated.

Because of observed results on ST concentrations and the relationships between ST, IGF-I and INS, regression analyses for these metabolic regulators were performed to evaluate changes in their plasma concentrations during this crucial period of metabolic adaptation to support lactation. Cubic polynomial regression curves (the highest order significant) were used to describe trends for all the regulators. Figure 4-1 shows the trend for ST during the transition period from d -21 to d 21 after parturition. Curves were not parallel as determined by heterogeneity test (Wilcox et al., 1990). Concentrations of ST depicted over time obtained from this regression curve indicated that cows treated with bST during both the prepartum and postpartum periods (TRT IV) were greater, bST treated cows also had elevated concentrations through the entire transition period. This

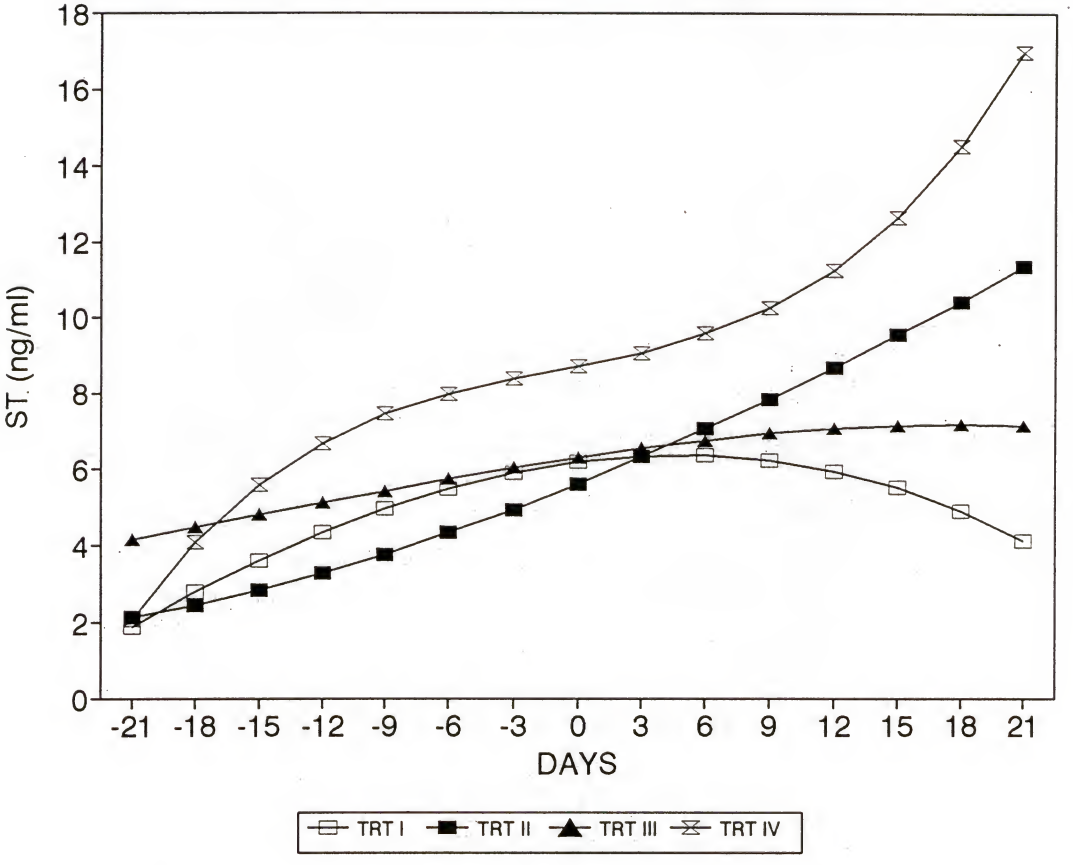

Figure 4-1. Regression Curves (cubic polynomial) Depicting Plasma Concentrations of ST During the Transition Period from d -21 to +21 d.

agreed with previous findings (Bachman et al.,1992; Simmons et al., 1994). Moreover, from these regressions it was observed that cows not treated during the prepartum period but treated after parturition (TRT II) had increased plasma concentrations of ST immediately after injections of bST were begun on d 0; they were maintained elevated during the postpartum period, whereas cows not treated at all (TRT I), as expected, showed a small increase as the time of parturition approached but concentrations decreased thereafter and remained low throughout the postpartum period. Cows treated during the prepartum period but not treated after parturition (TRT III) showed elevated plasma concentrations of ST during the prepartum period but concentrations were not maintained higher after parturition, which was after the injections had been discontinued. These changes in concentrations were as expected, and the magnitude of the increases in ST concentrations were appropriate for the amount of bST injected either prepartum or postpartum.

Regression curves for INS indicated that there were no differences among treatments during the prepartum period. All four treatment groups showed decreased plasma concentrations of INS as parturition approached and concentrations remained low thereafter, until d 15 when cows in treatment groups I, III, and IV but not II, began to show increased concentrations (figure 4-2). These results agreed with published literature that during late pregnancy there is an increased resistance to INS which can be observed as diminished sensitivity of several measures of whole body glucose utilization and decreased responsiveness of adipose tissue lipolysis and NEFA mobilization (Petterson et al., 1993,1994). Concentrations of INS declined around parturition (Malven et al.,

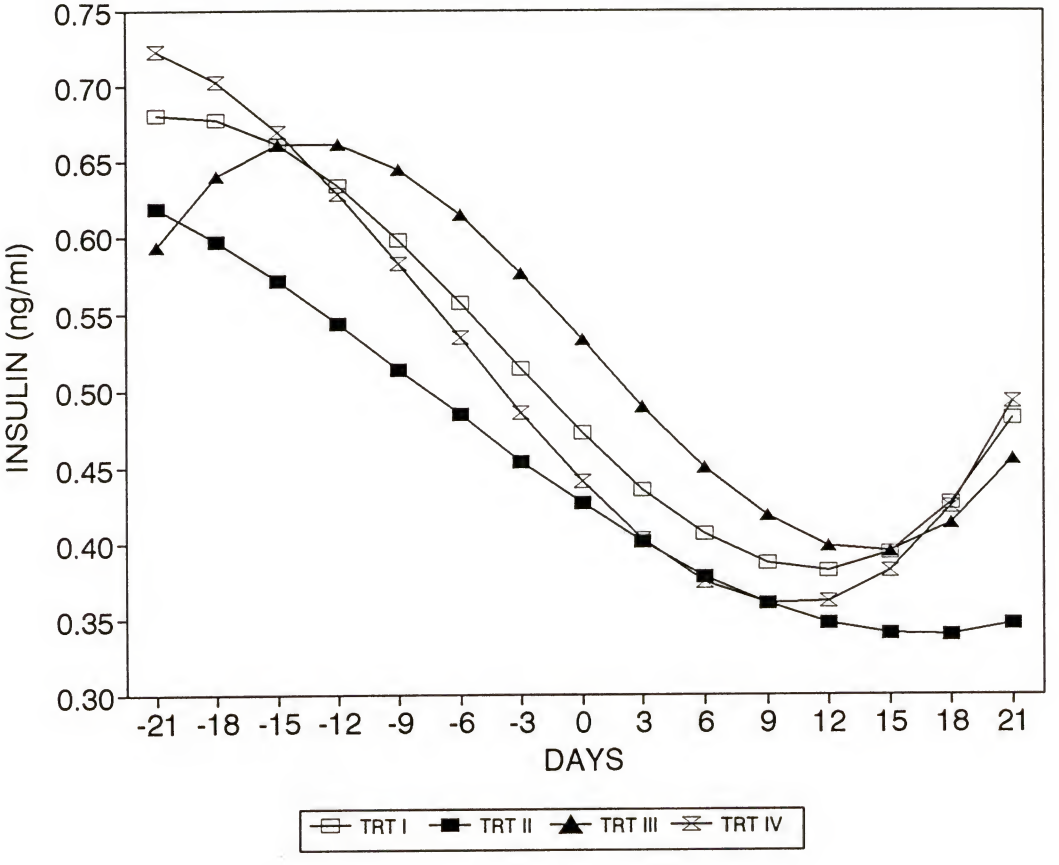

Figure 4-2. Regression Curves (cubic polynomial) Depicting Plasma Concentrations of INS During the Transition Period from d -21 to +21 d.

1987b). Increased concentrations of ST decreased the ability of INS to inhibit gluconeogenesis, inhibited actions of INS second messenger and altered INS uptake into the cell, and its degradation by inhibiting the INS protease necessary for normal actions of INS. All of these actions would favor production of glucose from available precursors and conservation of glucose for mammary use by shifting peripheral tissues to utilization of other substrates made available by actions of ST and INS.

Regression curves to depict plasma concentrations of IGF-I during the transition period also were plotted by using regression coefficients obtained from cubic regression analyses (figure 4-3). Curves were not parallel as denoted by the heterogeneity test (Wilcox et al., 1990). All treatment groups followed the same trend with high plasma concentrations of IGF-I during the prepartum period (d -21) and a continuous decrease through parturition with the lowest plasma concentrations observed during first 2 wk after parturition. However, prepartum bST- treated cows (TRT III and IV) had higher plasma concentrations of IGF-I during the prepartum period and during the postpartum period. From d + 15 postpartum plasma concentrations of IGF-I for cows in all four treatment groups began to increase. However, rate of increase in IGF-I concentrations was greatest for prepartum treated cows whether or not they were injected with bST after parturition (TRT III and TRT IV). Although cows in treatment group III had slightly higher plasma concentrations of IGF-I, rate of IGF-I increase after parturition was faster for the prepartum and postpartum bST treated cows (TRT IV). On the other hand, cows not injected during the prepartum period not only had the lowest plasma concentrations of IGF-I during the prepartum period but also on calving day. Also rate of increase after

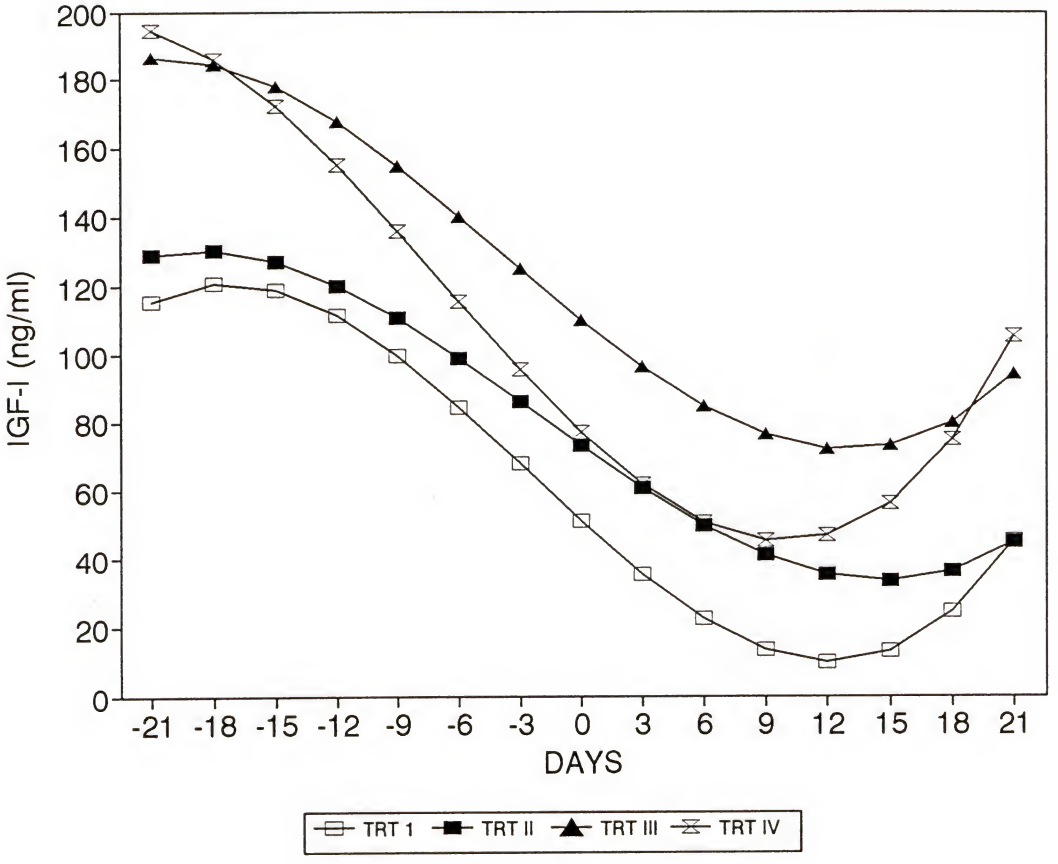

Figure 4-3. Regression Curves (cubic polynomial) Depicting Plasma Concentrations of IGF-I During the Transition Period from d -21 to +21 d.

parturition was lower for these groups of cows, whether or not they were injected with bST during the postpartum period. These results indicated a relationship between the increase of IGF-I concentrations after parturition and the rapid increase in DMI experienced by cows treated with bST during both prepartum and postpartum periods.

Circulating concentrations of IGF-I correlate well with ST secretion during postnatal life. Hepatic expression of IGF-I is responsive primarily to ST. Actions of ST are mediated in many cases (indirect actions) by IGF-I (Bauman, 1992). Thus, plasma concentrations of IGF-I are under control of ST; however, nutritional status plays a major role in the regulation of the somatotropic-axis. Thus, both hepatic and extrahepatic tissue synthesis of IGF-I may constitute a switch which promotes nutrient utilization for critical functions such as growth and lactation and would involve either paracrine or endocrine mechanisms (Jones and Clemmons, 1995; Phillips et al., 1990).

Results of the current study agreed with previous findings that treatment of cows with bST resulted in increased concentrations of IGF-I during the dry period (Bachman et al, 1992); during both early and late lactation (Cisse et al., 1991; Lucy et al., 1993; Staples et al., 1988), during later stages of lactation (Bauman, 1992; Dahl et al., 1993; Hadsell et al., 1993; Prosser et al., 1989; Zhao et al 1994); and when cows reached positive energy balance (Spicer et al., 1990). The response to bST seemed to be a general one across a diverse set of physiological states. Decreased plasma concentrations of IGF-I during the week before calving and during the first 2 wk after calving seemed to be a response to decreased DMI experienced by the cows during the last week prepartum, when DMI was reduced by one-third or more. Low nutrient balance, primarily energy,

appeared to be the major regulator of concentrations of IGFBP3 and responsiveness of IGF-I to exogenous ST before parturition (Simmons et al., 1994) and during early lactation (Armstrong and Benoit, 1996; Ronge et al., 1988; Spicer et al., 1990; Vicini et al., 1991).

Least squares analyses of variance for ST, INS and IGF-I during the overall postpartum period (d 0 to 65) are in table 4-9. A significant difference ($P<0.0005$) was detected for main effect of PPTRT for ST but not for INS or IGF-I. Additionally, the interaction PRETRT*diet was significant for both ST ($P < 0.0383$) and INS ($P < 0.0542$), whereas a significant two-factor interaction of PRETRT*PPTRT and a significant cubic effects of days were detected for INS but not the others. Least squares means and SE for hormones, growth factor and blood measures due to the effect of bST treatment during the postpartum period (d 0 to 65) are in table 4-10. As described already, during the transition period (d -21 to d +21) bST-treated cows had greater plasma concentrations of ST (TRT II and IV), whereas plasma concentrations of INS were similar across treatment groups. Plasma concentrations of IGF-I tended to follow the same pattern as ST but with less difference among treatment means.

Interestingly, although accumulated plasma concentrations of P_4 did not differ when analyzed by analyses of variance, least squares means shown in table 4-10 indicated that cows not injected at all during the experimental period had numerically higher accumulated concentrations of P_4 (23.38 ng/ml) during 65 d of lactation than cows not injected during the prepartum period but injected after parturition (19.64 ng/ml), and

Table 4-9. Least Squares Analyses of Variance for ST, INS, and IGF-I of Holstein Cows During Early Lactation (0-65 d).

		ST[1]		INS[2]		IGF-I[3]	
Source	df	MS	PR > F	MS	PR > F	MS	PR > F
Pretrt[4]	1	237.03	0.4587	0.25	0.4416	20584.35	0.2507
Diet	1	127.53	0.5861	0.05	0.7348	14316.44	0.3368
Pretrt * Diet	1	1947.96	0.0383	0.64	0.0542	21163.64	0.2442
PPtrt[5]	1	6161.62	0.0005	0.75	0.1876	3003.98	0.6584
Pretrt * PPtrt	1	13.43	0.8595	1.27	0.0885	729.24	0.8274
Diet * PPtrt	1	341.11	0.3748	0.84	0.1642	170.29	0.9161
Pretrt * Diet * PPtrt	1	938.77	0.1445	0.13	0.5852	7035.36	0.4994
Cow (Pretrt * Diet * Pptrt)	38	422.90	0.0001	0.42	0.0001	15127.59	0.0001
Days (D)[6]	1	261.05	0.0060	1.53	0.0001	238472.32	0.0001
D * D	1	158.43	0.0321	0.01	0.4627	1110.70	0.2554
D * D * D	1	32.19	0.3337	0.02	0.2815	4545.01	0.0215
Error	1239	34.42		0.02		857.70	

[1]Somatotropin. [2]Insulin. [3]Insulin-like Growth Factor-I.
[4]Prepartum treatment 0,5 mg bST/d.
[5]Postpartum treatment 0,5 mg bST/d.
[6]Sums of Squares Type I for all D terms; Type III for others.

Table 4-10. Least Squares Means and SE for Hormones, Growth Factor, and Blood Measures of Holstein Cows During early Lactation (0-65 d).

Blood Measures	Treatments[1]											
	I			II			III			IV		
ST (ng/ml)	5.44	±	0.34	9.73	±	0.33	6.10	±	0.33	10.81	±	0.34
INS (ng/ml)	0.48	±	0.01	0.37	±	0.01	0.45	±	0.01	0.46	±	0.01
IGF-I (ng/ml)	61.44	±	1.71	63.01	±	1.66	68.15	±	1.64	72.80	±	1.71
PRL (ng/ml)	11.15	±	0.41	10.54	±	0.38	12.13	±	0.38	10.14	±	0.40
T_4 (ng/ml)	61.21	±	1.01	61.48	±	0.97	56.07	±	0.96	70.99	±	1.00
T_3 (pg/ml)	539.47	±	17.16	471.25	±	25.00	519.08	±	16.81	536.68	±	69.00
P_4 (ng/ml)	23.38	±	8.68	19.64	±	0.66	15.96	±	0.65	18.48	±	0.68
HMT%	27.60	±	0.09	26.76	±	0.09	27.45	±	0.09	26.94	±	0.09
PP%	7.31	±	0.04	7.19	±	0.04	7.26	±	0.04	7.36	±	0.04

[1]Treatment (Prepartum, Postpartum); Treatment I= 0,0 mg bST/d; II=0,5 mg bST/d; III=5,0 mg bST/d; IV=5,5 mg bST/d.

higher than cows injected during the prepartum period and injected (18.48 ng/ml) or not injected (15.96 ng/ml) during the postpartum period. Regression analyses were performed on accumulated P_4. Regression curves were plotted for the four treatment groups using regression coefficients from cubic polynomial regression analyses (figure 4-4). Heterogeneity test indicated curves were not parallel. From parturition until 42 d postpartum all treatment groups displayed the same general pattern with moderate increases in accumulated P_4, which is indicative of some ovarian activity. However, for times greater than d 42 postpartum curves depicting accumulated plasma concentration of P_4 for cows not injected before parturition separated and appeared to be different, but were not by test of heterogeneity, from those of cows injected with bST during the prepartum period. However, cows not injected during the prepartum period but injected after parturition had greater accumulated P_4 than cows treated before calving either injected or not with bST after parturition.

Ovarian activity after parturition has been related to plasma concentrations of P_4. Cows with a plasma concentration of 1 ng/ml P_4 during two consecutive samples within a week when 3 samples per week are collected are considered as cycling cows. Thus, results from this study indicated that cows not treated with bST at all during the experiment returned to ovarian activity within 27.4 d after partition but 10% of cows within this group had not cycled by d 65, which was the last day of the experiment. Cows not injected during the prepartum period but injected after parturition demonstrated ovarian activity within 29.4 d but 25% of these cows did not show ovarian activity at all. Cows treated with bST during the prepartum period, but not injected after parturition,

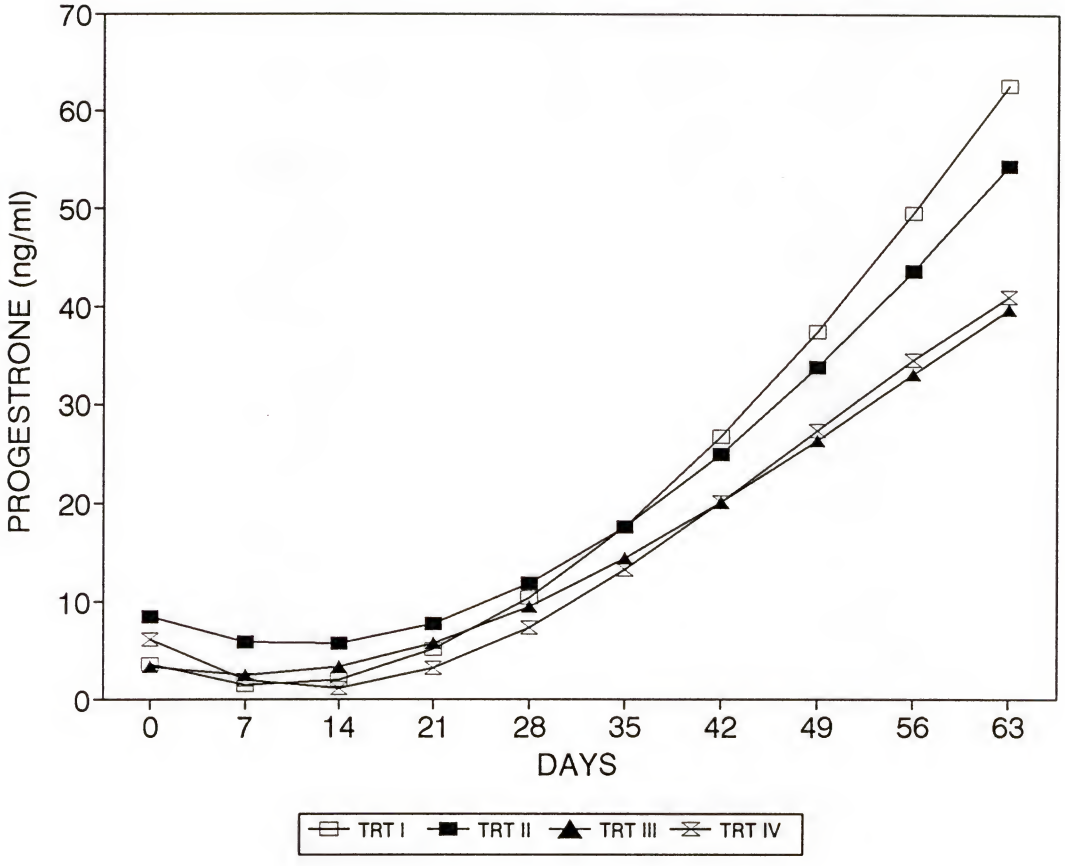

Figure 4-4. Regression Curves (cubic polynomial) Depicting Accumulated Plasma Concentrations of Progesterone During the Postpartum Period from 0-65 d.

showed ovarian activity by 29.4 d after parturition but 25% of cows did not show ovarian activity. Cows injected with bST both prepartum and postpartum showed ovarian activity by 27.5 d after parturition and 18 % of cows did not return to activity by 65 d of lactation.

Although the number of days to return to ovarian activity were numerically similar and no significant differences by heterogeneity test were observed for regressions of accumulated plasma concentrations of P_4 among treatment groups, the percentage of cows that did not return to activity by d 65 postpartum appeared to differ for cows in TRT I compared to cows in TRT II, III and IV. However, many factors could have influenced results observed during the study. Clearly, the number of cows assigned to each treatment group (12 cows/group) was too small to detect significant differences, but the trend is interesting. The growth factor IGF-I and the hormone ST are known to stimulate ovarian follicular development or function (Lucy et al., 1993). Cows injected with bST returned to first estrus 9 d earlier than controls (Stanisiewski et al., 1992). Treatment with bST had increased number of antral follicles (Gong et al., 1991, 1993a,b). Energy and other glucogenic metabolites, including protein, have been associated with IGF-I mRNA transcription and translation. Thus, an increase in the energy balance is associated with an increase in concentrations of IGF-I during early lactation. This increase is associated with an increase of P_4 secretion during diestrus of the first and second estrous cycles, but increased milk yield is associated with decreased serum concentrations of IGF-I and decreased energy balance (Spicer et al., 1990). Peak concentrations of P_4 during the first estrous cycle occurring 32 d after calving were twice as great in cows in positive energy balance than in cows in negative energy balance.

Decreased DMI during week before calving and wk 1 and 2 after calving likely is importantly associated with the low P_4 secretion and cows not returning to ovarian activity after parturition. Correlation coefficient indicated a low (r = -0.20) and negative relationship between energy balance and plasma concentrations of P_4, whereas correlation between ST and P_4 was positive and significant (r = 0.07) and between IGF-I and P_4 negative but significant (r = -0.05).

Conclusions

Results of this study suggest that injections of 5 mg of bST/d before and after parturition increased plasma concentrations of ST and IGF-I with a small increase in milk production and in DMI at a time when there are high nutrients demands. These responses did not affect plasma concentrations of INS and P_4 adversely during the early postpartum period. Furthermore, nutrient balance appeared to be the major regulator of IGF-I secretion and responsiveness of the cows to exogenous bST before parturition and during early lactation. Therefore, evidence was obtained that causing small changes in circulating hormones, growth factor and glucose concentrations had beneficial effects on the cow during the transition period that were seen as increased DMI, milk yield and efficiency of milk production during the rise of lactation to the peak. No strong adverse effects of bST treatment were evident during either the prepartum and postpartum periods.

CHAPTER 5
GENERAL CONCLUSIONS

Bovine Somatotropin (bST) is one of the first biotechnology derived products available for use in animal production systems. bST is a protein hormone, which many investigations have demonstrated to improve milk yield and lactation persistency for all breeds of dairy animals examined. However, quality of management is the major factor affecting magnitude of milk response to bST. Productive efficiency of dairy cows has been defined as the yield of milk and milk components relative to the nutritional cost of maintenance, lactation, and of returning the cow to the level of body condition that existed before the onset of the lactation. Although this can be used as a measure of efficiency during lactation, the costs of raising animals to the age where they can enter the dairy herd must be factored into the short-term and long-term profitability of a dairy animal. Because dairy farmers must invest capital, time and feed in order to raise replacements for their dairy herds, raising heifer replacements will increase profitability only when it is accomplished more efficiently with reduced costs. One way to decrease the cost of raising replacements is by decreasing the time for them to reach first parturition.

From this study it can be concluded that heifers raised at moderate or rapid growth rates between 90 to 365 d of age had greater body weight when they were fed a high plane

of nutrition during the prepubertal growth period from 90 to 220 d, and during the peripubertal growth period from 220 to 365 d of age. Injections of bST during the peripubertal growth period increased growth rates irrespective of whether the heifers were fed diets for normal or rapid growth rates. Therefore, heifers increased their body size and as result maturation of the reproductive system likely occurred earlier, and thus, these fast growing heifers could be inseminated for the first time at a younger age. Additionally, because research has shown the economic benefits of first parturition between 22-24 mo of age, use of bST injections during the peripubertal growth period suggested that bST may be a useful and alternative management tool in heifer raising that will allow the farmer to achieve target body weights and ages at first calving. Furthermore, milk yields of heifers did not differ due to plane of nutrition or bST treatment during the peripubertal growth period. However, ADGs during the peripubertal growth period were significantly and positively associated with actual or 305-d and total milk yields. Thus, based on results that described improved growth performance and body composition when bST was used, it is important to consider that bST could be used as a positive growth regulator in heifer rearing programs on commercial dairy farms. Increased rate of skeletal growth and reduced adipose tissue deposition of dairy heifers typically occurs during the prepubertal growth period, thus use of bST may be beneficial, especially if it were used around puberty or during the pregnancy growth phase. This could have beneficial effects on milk production during first lactation. Growth promoting and repartitioning effects of exogenous bST already have been demonstrated, thus manipulation of growth by use of bST as a repartitioning agent should be included within

the heifer raising program on the dairy farm as an integral part of dairy operations.

On the other hand, a major objective of dairy farmers is to keep cows producing milk throughout the year. Importantly, plane of nutrition during lactation can have a significant effect on milk yield and on timing of the onset of normal ovarian activity postpartum. In cows, lactation results in a series of metabolic changes which decreases rate of fatty acid synthesis in adipose tissue while at the same time causing an increase in the mobilization of body tissues, especially during early lactation. These adaptations occur during the latter stages of pregnancy and during the first several weeks of lactation (termed the "transition period") to facilitate the preferential use of lipogenic precursors by the mammary gland. Hormones responsible for these adaptations were shown to be INS, PRL, and ST. Collectively, data have demonstrated that under certain physiological conditions such as lactation, alterations in energy metabolism may influence hypothalamic-pituitary function. Because ST is a homeorhetic controller that shifts the partitioning of nutrients, and because increased voluntary intake of DM has been seen in bST supplemented cows, and this increased DMI persists during the time bST is supplemented, results of this study indicated that injections of low doses of bST during the early postpartum period increased rate of increase in DMI after parturition and thus, greater amount of nutrients and energy were ingested to support the lactation phase. Furthermore, greater DMI when cows were injected with bST prepartum and postpartum allowed them to recover BW and BCS at a faster speed and to reach positive energy balance in less time during the lactation, while they were producing numerically, but not significantly, greater amounts of milk and 4% FCM. Finally, less BW loss during the

transition period after calving and slightly greater milk production during this period resulted in more efficient production of 4% FCM. Although cow numbers were too limited to critically evaluate the effects of the four treatment combinations on health and calving problems, no apparent differences were noted. It appears that injections of bST could be used during the prepartum and/or the postpartum periods, but indications are that treating cows during both periods had positive effects that will improve efficiency of milk production and perhaps improve overall yields of milk, although the latter conclusion must be proven with greater numbers of cows and for the entire lactation.

In addition to that, result of this study suggest that injections of 5 mg of bST/d before and after parturition increased plasma concentrations of ST and IGF-I with a small increase in DMI and milk production. Furthermore, nutrient balance appeared to be the major regulator of IGF-I secretion and responsiveness of the cows to exogenous bST before parturition and during early lactation. Therefore, evidence was obtained that causing small changes in circulating hormone, growth factor and glucose concentrations had beneficial effects on the cow during the transition period; the beneficial effects were seen as increased DMI, milk yield and efficiency of milk production during the rise of lactation to peak milk yield. No strong adverse effects of bST treatment were evident during either the prepartum and/or the postpartum period that would preclude its use during these time periods.

APPENDIX

REGRESSION COEFFICIENTS FOR VARIOUS PERFORMANCE MEASURES OF HOLSTEIN COWS DURING EARLY LACTATION

Table 1. Regression Coefficients (5th order) for DMI of Holstein Cows During Early Lactation[1].

Trt	BO	B1	±SE	B2	±SE	B3	±SE	B4	±SE	B5	±SE
I (0,0)	13.144340	1.63713295	0.24744990	-0.11912725	0.02442282	0.00430692	0.00097531	-0.00007095	0.00001683	0.00000043	0.00000010
II (0,5)	6.433739	1.13195077	0.23712219	-0.06699250	0.02341062	0.00208351	0.00093544	-0.00003054	0.00001616	0.00000017	0.00000010
III (5,0)	10.406860	1.27184761	0.23742588	-0.07961456	0.02343436	0.00235758	0.00093615	-0.00003169	0.00001617	0.00000016	0.00000010
IV (5,5)	11.070310	0.79017945	0.24761649	0.00374571	0.02444676	-0.00092299	0.00097691	0.00001851	0.00001688	-0.00000001	0.00000010

[1]Lactation from 0-65 d.

Table 2. Regression Coefficients (5th order) for Energy Balance of Holstein Cows During Early Lactation [1]

Trt	BO	B1	±SE	B2	±SE	B3	±SE	B4	±SE	B5	±SE
I (0,0)	-18.0986	-1.63989033	1.21691541	0.19925425	0.12010716	-0.00672103	0.00479641	0.00009397	0.00008279	-0.00000047	0.00000051
II (0,5)	-25.7616	-1.29335959	1.16641037	0.16007387	0.11518343	-0.00500978	0.00460371	0.00006964	0.00007956	-0.00000038	0.00000049
III (5,0)	-12.7846	-5.54381110	1.16761905	0.59253740	0.11524608	-0.02234512	0.00460383	0.00036458	0.00007952	-0.00000217	0.00000049
IV (5,5)	-27.1462	2.15532430	1.21815041	-0.08077741	0.12029234	0.00147330	0.00480766	-0.00001138	0.00008307	0.00000003	0.00000052

[1]Lactation from 0-65 d.

Table 3. Regression Coefficients (3rd order) for Body Weight Change of Holstein Cows During Early Lactation [1]

Trt	BO	B1	±SE	B2	±SE	B3	±SE
I (0,0)	459.5140	-1.77217110	0.23069406	0.03326580	0.00832267	-0.00020820	0.00008502
II (0,5)	467.5499	-2.02906800	0.22820873	0.02516110	0.00814330	-0.00004820	0.00008304
III (5,0)	451.8247	-2.55997490	0.22323583	0.05879420	0.00804817	-0.00041090	0.00008228
IV (5,5)	465.8689	-0.43409120	0.23020984	0.01405010	0.00833445	-0.00015760	0.00008541

[1]Lactation from 0-65 d.

Table 4. Regression Coefficients (3rd order) for BCS of Holstein Cows During Early Lactation[1].

Trt	BO	B1	±SE	B2	±SE	B3	±SE
I (0,0)	3.312860	-0.02825550	0.00305640	0.00062822	0.00011127	-0.00000442	0.00000114
II (0,5)	3.593138	-0.36035487	0.00293765	0.00077130	0.00010727	-0.00000521	0.00000110
III (5,0)	3.475546	-0.02647449	0.00293324	0.00055145	0.00010697	-0.00000359	0.00000110
IV (5,5)	3.438555	-0.01735411	0.00306367	0.00028702	0.00011173	-0.00000112	0.00000115

[1]Lactation form 0-65 d.

Table 5. Regression Coefficients (5th order) for Milk Yield of Holstein Cows During Early Lactation[1].

Trt	BO	B1	±SE	B2	±SE	B3	±SE	B4	±SE	B5	±SE
I (0,0)	16.369280	3.35906035	0.42357691	-0.21805261	0.03957836	0.00698246	0.00152911	-0.00010509	0.00002583	0.00000059	0.00000016
II (0,5)	13.485580	2.40376408	0.40615280	-0.12997286	0.03797969	0.00332069	0.00146851	-0.00003965	0.00002483	0.00000018	0.00000015
III (5,0)	14.807500	3.38571752	0.40615280	-0.21974940	0.37979690	0.00696122	0.00146851	-0.00010312	0.00002483	0.00000057	0.00000015
IV (5,5)	17.753180	1.57129589	0.42437867	-0.01702576	0.03969233	-0.00059247	0.00153495	0.00001441	0.00002595	-0.00000009	0.00000016

[1]Lactation from 0-65 d.

Table 6. Regression Coefficients (5th order) for 4% FCM of Holstein Cows During Early Lactation [1]

Trt	BO	B1	±SE	B2	±SE	B3	±SE	B4	±SE	B5	±SE
I (0,0)	17.441760	3.31210078	0.49004180	-0.23355420	0.04578873	0.00787489	0.00176905	-0.00012271	0.00002988	0.00000071	0.00000018
II (0,5)	26.051340	1.90002441	0.46988361	-0.11460180	0.04393921	0.00299844	0.00169894	-0.00003520	0.00002872	0.00000015	0.00000018
III (5,0)	15.669750	3.68651034	0.46988361	-0.24699335	0.04393921	0.00756207	0.00169894	-0.00010717	0.00002872	0.00000057	0.00000018
IV (5,5)	21.856630	1.17825549	0.49096936	0.00195728	0.04592058	-0.00114903	0.00177581	0.00002271	0.00003003	0.00000013	0.00000018

[1]Lactation from 0-65 d.

REFERENCES

Abribat, T. H., H. Lapierre, P. Dubreuil, G. Pelletier, P. Gaudreau, P. Brazeau, and D. Petitclerc. 1990. Insulin-like growth factor-1 concentration in Holstein female cattle: variations with age, stage of lactation and growth hormone-releasing factor administration. Domest. Anim. Endocrinol. 7:93-102.

Armstrong, J. D., and A. M. Benoit. 1996. Paracrine, autocrine, and endocrine factors that mediate the influence of nutrition on reproduction in cattle and swine: an in vivo, IGF-1 perspective. J. Anim. Sci. 74:18-35.

Armstrong, D. T., and W. Hansel. 1956. The effect of age and plane of nutrition on growth hormone and thyrotropic hormone content of pituitary glands of Holstein heifers. J. Anim. Sci. 15:640-647.

Bachman, K. C., D. H. Wilfond, H. H. Head, C. J. Wilcox, and M. Singh. 1992. Milk yields and hormone concentrations of Holstein cows in response to Sometribove (Somatotropin) treatment during the dry period. J. Dairy Sci. 75:1883-1890.

Baldwin, R. L., and L. Suchet. 1974. Hormonal actions on mammary metabolism. J. Dairy Sci. 58:1033-1041.

Bass, J. J., and P. D. Gluckman. 1990. Regulation of growth by the growth hormone axis. Proc. N. Z. Soc. Anim. Prod. 50:73-79.

Bauman, D. E. 1992. Bovine somatotropin: Review of an emerging animal technology. J. Dairy Sci. 75:3432-3451.

Bauman, D. E., and C. L., Davis. 1975. Regulation of lipid metabolism. *In*: Digestion and metabolism in the ruminant. I. W. McDonald, and A. C. I. Warner, ed. Univ. New England Publ. Unit., Armidale, New South Wales, Australia. p.496-505.

Bauman, D.E., and J. M. Elliot. 1983. Control of nutrient partitioning in lactating ruminants. In: T. B. Mephan (Ed.). Biochemistry of Lactation Elsevier, Amsterdam, The Netherlands. p437.

Bauman, D. E., P. J. Eppard, M. J. DeGeeter, and G. M. Lanza. 1985. Responses of high producing dairy cows to long-term treatment with pituitary somatotropin and recombinant somatotropin. J. Dairy Sci. 68:1352-1361.

Bauman, D. E., C. J. Peel, W. D. Steinhour, P. J. Reynolds, H. F. Tyrrell, A. C. G. Brown, and G. L. Haaland. 1988. Effect of bovine somatotropin on metabolism of lactating dairy cows: influence on rates of irreversible loss and oxidation of glucose and nonesterified fatty acids. J. Nutr. 118:1031-1040.

Bauman, D. E., and R. G. Vernon. 1993. Effects of exogenous bovine somatotropin on lactation. Annu. Rev. Nutr. 13:437-445.

Bell, A .W. 1995. Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. J. Anim. Sci. 73:2804-2819.

Bernal Santos, G. 1982. Changes in glucose and energy homeostasis with onset of lactation. M. S. Thesis. Cornell Univ., Ithaca, NY.

Bertics, S. J., R. R. Grummer, C. Cadorniga-Valino, and E. E. Stoddard. 1992. Effect of prepartum dry matter intake on liver triglyceride concentration and early lactation. J. Dairy Sci. 75:1914-1922.

Binelli, M., W. K. Vanderkooi, L .T. Chapin, M. J. VandeHaar, J. D. Turner, W. M. Moseley, and H. A. Tucker. 1995. Comparison of growth hormone-releasing factor and somatotropin: body growth and lactation of primiparous cows. J. Dairy Sci. 78:2129-2139.

Boisclair, Y., D. G. Grieve, J. B. Stone, O. B. Allen, and G. K. Macleod. 1986. Effect of prepartum energy, body condition, and sodium bicarbonate on production of cows in early lactation. J. Dairy Sci. 69:2636-2647.

Bonczek. R. R., C. W. Young, J. E. Wheathon, and K. P. Miller. 1988. Responses to somatotropin, insulin, prolactin, and thyroxine to selection for milk yield in Holsteins. J. Dairy Sci. 71:2470-2476.

Breier, B. H., J. J. Bass, J. H. Butler, and P. D. Gluckman. 1986. The somatotropic axis in young steers: Influence of nutritional status on pulsatile release of growth hormone and circulating concentrations of insulin like growth factor-1. J. Endocrinol. 111:209-217.

Breier, B. H., P. D. Gluckman, and J. J. Bass. 1988. The somatotropic axis in young steers: Influence of nutrition and estradiol-17B on hepatic high and low affinity somatotropic binding sites. J. Endocrinol. 116:169-174.

Brody, S. 1945. Bioenergetics and Growth. Reinhold Pub. Corp., New York, NY.

Brumby, P. J. 1959. The influence of growth hormone on growth in young cattle. N. Z. J. Agric. Res. 2:683-688.

Buskirk, D. D., D. B. Faulkner, W. L. Hurley, D. J. Kesler, F. A .Ireland, T. G. Nash, J. C. Castree, and J. L. Vicini. 1996. Growth, reproductive performance, mammary development, and milk production of beef heifers as influenced by prepubertal dietary energy and administration of bovine somatotropin. J. Anim. Sci. 74:2649-2662.

Capuco, A. V., J. J. Smith, D. R. Waldo, and C. E. Rexroad, Jr. 1995. Influence of prepubertal dietary regimen on mammary growth of Holstein heifers. J. Dairy Sci. 78:2709-2725.

Carlsson, L. M. S., R. G. Clark, A. Skottner, and C. A. F. Robinson. 1989. Growth hormone and growth in diabetic rats: effects of insulin and insulin-like growth factor-I infusions. J. Endocrinol. 122:661-671.

Chalupa, W., and D. T. Galligan. 1989. Nutritional implications of somatotropin for lactating cows. J. Dairy Sci. 72:2510-2518.

Chase, L. E. 1993. Developing nutrition program for high producing dairy herds. J. Dairy Sci. 76:3287-3293.

Chew, B. P., J. R. Eisenman, and T.S. Tanaka. 1984b. Arginine infusion stimulates prolactin, growth hormone, insulin and subsequent lactation in pregnant dairy cows. J. Dairy Sci. 67:2507-2518.

Chew, B. P., R. E. Erb, and P. V. Malven. 1976. Assay of prolactin after freezing cow's milk. J. Dairy Sci. 60:981-984.

Chew, B. P., F. R. Murdock, R. E .Riley, and J. K. Hillers. 1983. Influence of prepartum dietary crude protein on growth hormone, insulin, reproduction, and lactation of dairy cows. J. Dairy Sci. 67:270-275.

Chew, B. P., F. R. Murdock, R. E. Riley, and J. K. Hillers. 1984a. Influence of prepartum dietary crude protein on growth hormone, insulin, reproduction and lactation of dairy cows. J. Dairy Sci. 67:270.

Chilliard, Y. 1989. Long-term effects of recombinant bovine somatotropin (rBST) on dairy cow performances: a review. p. 61. In: Use of somatotropin in livestock production. K. Sejrsen, M. Vestergaard, and A. Neimann-Sorensen, ed. Elsevier Appl. Sci., New York, NY.

Chilliard, Y. 1993. Dietary fat and adipose tissue metabolism in ruminants, pigs and rodents. A review. J. Dairy Sci. 76:3897-3931.

Chilliard, Y., M. Cisse, R. Lefaivre, and B. Remond. 1991. Body composition of dairy cows according to lactation stage, somatotropin treatment, and concentrate supplementation. J. Dairy Sci. 74:3103-3116.

Cisse, M., Y .Chilliard, V. Coxam, M. J. Davicco, and B. Remond. 1991. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 2. Plasma hormones and metabolites. J. Dairy Sci. 74:1382-1394.

Clemmons, D. R., and L. E. Underwood. 1991. Nutritional regulation of IGF-I and IGF binding proteins. Annu. Rev. Nutr. 11:393-399.

Coppock, C. E., C. H. Noller, S. A. Wolfe, C. J. Callhan, and J. S. Baker. 1972. Effect of forage concentrate ratio in complete feeds fed ad libitum on feed intake prepartum and the occurrence of abomasal displacement in dairy cows. J. Dairy Sci. 55:783-792.

Crooker, B. A., M. A. McGuire, W. S. Cohick, M. Harkins, D. E. Bauman, and K. Sejrsen. 1990. Effect of dose of bovine somatotropin on nutrient utilization in growing dairy heifers. J. Nutr. 120:1256-1264.

Daccarett, M. G., E J. Bortone, D. E. Isbell, and J. L. Morrill. 1993. Performance of Holstein heifers fed 100% or more of National Research Council Requirements. J. Dairy Sci. 76:606-614.

Dado, R. G., and M. S. Allen. 1993. Continuous computer acquisition of feed and water intake, chewing, reticular motility and ruminal pH of cattle. J. Dairy Sci. 76:1589-1600.

Dahl, G. E., L. T. Chapin, M. S. Allen, W. M. Moseley, and H. A. Tucker. 1991. Comparison of somatotropin and growth hormone-releasing factor on milk yield, serum hormones, and energy status. J. Dairy Sci. 74:3421-3428.

Dahl, G. E., L. T. Chapin, W. M. Moseley, and H. A. Tucker. 1993. Galactopoietic effects of recombinant somatotropin and growth hormone-releasing factor in dairy cows. J. Dairy Sci. 76:1550-1558.

Daughaday, W. H. 1981. The adenohypophysis. In: R. H. Williams (Editor), Textbook of Endocrinology, 6th edn. W. B. Saunders, Philadelphia, PA, pp. 87-92.

Daughaday, W. H., I. K. Mariz, and S. L. Blethen. 1980. Inhibition of access to bound somatomedin to membrane receptor and immunobinding site: a comparison of radioreceptor and radioimmunoassay of somatomedin in native and acid ethanol extract serum. J. Clin. Endocrinol. & Metab. 51:781-788.

Davis, R. S., and R. J. Collier. 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J. Dairy Sci. 68:1041-1046.

Davis, A. J., I. R. Fleet, J. A. Goode, M. H. Hamon, F. M. Maule-Walker, and M. Peaker. 1979. Changes in mammary function at the onset of lactation in the goat: correlation with hormonal changes. J. Physiol. 188:33-43.

De Boer G., and J. J. Kennelly. 1989. Effect of somatotropin injection and dietary protein concentration on milk yield, and kinetics of hormones in dairy cows. J. Dairy Sci. 72:419-428.

Dehoff, M. H., R. G. Elgin, R. J. Collier and D. R. Clemmons. 1988. Both type I and II insulin-like growth factor receptor binding increase during lactogenesis in bovine mammary tissue. Endocrinology. 122:2412-2417.

Di Marco, O. N., R. L. Baldwin, and C. C. Calvert. 1987. Relative contributions of hyperplasia and hypertrophy to growth in cattle. J. Anim. Sci. 65:150-157.

Dirksen, G. U., H. G. Liebich, and E. Mayer. 1985. Adaptative changes of the ruminal mucosa and their functional and clinical significance. Bovine Pract. 20:116-127.

Drackley, J. K., M. J. Richard, D. C. Beitz, and J. W. Young. 1992. Metabolic changes in dairy cows with Ketonemia in response to feed restriction and dietary 1,3-Butanediol. J. Dairy Sci. 75:1622-1634.

Drackley, J. K., J. J. Veenhuzen, M. J. Richard, and J. W. Young. 1991. Metabolic changes in blood and liver of dairy cows during either feed restriction or administration of 1,3-Butanediol. J. Dairy Sci. 74:4254-4264.

Dunshea, F. R., A. W. Bell, and T. E. Trigg. 1988. Relations between plasma non-esterified fatty acid metabolism and body tissue mobilization during chronic undernutrition in goats. Br. J. Nutr. 60:633-645.

Early, R. J., B. W. McBride, and R. O. Ball. 1990. Growth and metabolism in somatotropin-treated steers: III. Protein synthesis and tissue energy expenditures. J. Anim. Sci. 68:4153-4159.

Eastridge, M. L., and D. L. Palmquist. 1988. Supplementary energy as calcium soaps beginning at two weeks of lactation. J. Dairy Sci. 71 (Suppl.1):254 (Abstr.).

Echtemkamp, S. E., L. J., Spicer, K. E. Canning, S. F., and J. M. Hammond. 1990. Concentrations of insulin-like growth factor-I in blood and ovarian follicular fluid in cattle selected for twins. Biol. Reprod. 43:8-14.

Emery, R. S. 1993. Energy needs of dry cows. In: Proc. Tri-State Dairy Nutr. Conf. Ohio State Univ., Michigan State Univ., and Purdue Univ., Ft. Wayne, IN. p35.

Enright, W. J. 1989. Effects of administration of somatotropin on growth, feed efficiency and carcass composition of ruminants: a review. In: K. Sejrsen, M. Vestergaard, and A. Neimann-Sorensen (Ed.) Use of Somatotropin in livestock production. Elsevier Appl. Sci., London, England. p.132-154.

Enright, W. J., L. T. Chapin, W. M. Moseley, S. A. Zinn, M. B. Kamdar, L. F. Krabill, and H. A. Tucker. 1989. Effects of infusions of various doses of bovine growth hormone-releasing factor on blood hormones and metabolites in lactating Holstein cows. J. Endocrinol. 122:671-679.

Erb, R. E. 1976. Hormonal control of mammogenesis and onset of lactation in cows - a review. J. Dairy Sci. 60:155-169.

Esteban, E., P. H. Kass, L. D. Weaver, J. D. Rowe, C. A. Holmberg, C. E. Franti, and H. F. Troutt. 1994. Interval from calving to conception in high producing dairy cows treated with recombinant bovine somatotropin. J. Dairy Sci. 77:2549-2561.

Faulkner, A., and H. T. Pollock. 1990. Metabolic responses to euglycaemic hyperinsulinaemia in lactating and non-lactating sheep in vivo. J. Endocrinol. 124:59-68.

Foldager, J., and K. Sejrsen. 1982. Nutrition of replacement heifers affects mammary development and their ability to produce milk. *In*: Proc. 12th World Congr. Dis. Cattle World Assoc. Buiatrics. Utrecht, Netherlands. p.451.

Foldager, J., and K. Sejrsen. 1987. Mammary gland development and milk production in dairy cows in relation to feeding and hormone manipulation during rearing. In: Research in Cattle Production Danish Status and Perspectives. Landhusholdningsselskabets Forlag, Copenhagen, Denmark. p.102.

Forbes, J. M. 1968. The physical relationships of the abdominal organs in the pregnant ewe. J. Agric. Sci. (Camb). 70:171-177.

Forbes, J. M. 1986. The effects of sex hormones, pregnancy and lactation on digestion, metabolism, and voluntary food intake. In: L. P. Michigan, W. L. Grovum, and A. Dobson (Ed.) Control of Digestion and Metabolism in Ruminants. Prentice-Hall, Englewood Cliffs, NJ. p.420-435.

Forsyth, I. A. 1986. Variation among species in the endocrine control of mammary growth and function: the roles of prolactin, growth hormone, and placental lactogen. J. Dairy Sci. 69:886-892.

Forsyth, I. A. 1996. The insulin-like growth factor and epidermal growth factor families in mammary cell growth in ruminants: Action and interaction with hormones. J. Dairy Sci. 79:1085-1096.

Fox, D. G., and J. R. Black. 1984. A system for predicting body composition and performance in growing cattle. J. Anim. Sci. 58:725-734.

Fronk, T. J., L. H. Schultz, and A. R. Hardie. 1980. Effect of dry period overconditioning on subsequent metabolic disorders and performance of dairy cows. J. Dairy Sci. 63:1080-1090.

Gagliostro, G., and Y. Chilliard. 1991. Duodenal rapeseed oil infusion in early and midlactation cows. 2. Voluntary intake, milk production, and composition. J. Dairy Sci. 74:499-509.

Gallo, G. F., and E. Block. 1990a. Effects of recombinant bovine somatotropin on nutritional status of dairy cows during pregnancy and of their calves. J. Dairy Sci. 73:3266-3275.

Gallo, G. F., and E. Block. 1990b. Effects of recombinant bovine somatotropin on nutritional status and liver function of lactating dairy cows. J. Dairy Sci. 73:3276-3286.

Garcia-Gavidia, A. N. 1995. Growth rates of Holstein heifers fed two planes of nutrition and treated with bST during the peripubertal period. MS Thesis. University of Florida.

Gardner, R. W., J. D. Schuh, and L. G. Vargus. 1977. Accelerated growth and early breeding in Holstein heifers. J. Dairy Sci. 60:1941-1952.

Garnsworthy, P. C., and G. P. Jones. 1987. The influence of body condition at calving and dietary protein supply on voluntary food intake and performance in dairy cows. Anim. Prod. 44:347-346.

Garnsworthy, P. C., and J. H. Topps. 1982. The effect of body condition of dairy cows at calving on their food intake and performance when given complete diets. Anim. Prod. 35:113-119.

Gearhart, M. A., C. R. Curtis, H. N. Ebb, R. D. Smith, C. J. Sniffen, L. E. Chase, and M. D. Cooper. 1990. Relationship of changes in condition score to cow health in Holsteins. J. Dairy Sci. 73:3132-3141.

Gochman, N., and J. M. Schmitz. 1972. Application of a new peroxide indicator reaction to the specific, automated determination of glucose with glucose oxidase. Clin. Chem. 18:943-950.

Gong, J. G., T. Bramley, and R. Webb. 1991. The effect of recombinant bovine somatotropin on ovarian function in heifers: follicular populations and peripheral hormones. Biol. Reprod. 45:941-949.

Gong, J. G., T. A. Bramley, and R. Webb. 1993a. The effect of recombinant bovine somatotropin on ovarian follicular growth and development in heifers. J. Reprod. Fertil. 97:247-255.

Gong, J. G., T. A. Bramley, I. Wilmut, and R. Webb. 1993b. Effect of recombinant bovine somatotropin on the superovulatory response to pregnant mare serum gonadotropin in heifers. Biol. Reprod. 48:1141-1149.

Grant, R. J., and J. L. Albright. 1995. Feeding behavior and management factors during the transition period in dairy cattle. J. Anim. Sci. 73:2791-2803.

Grinari, J. M., M. A. McGuire, D. A. Dwyer, D. E. Bauman, and D. Palmquist. 1997. Role of insulin in the regulation of milk fat synthesis in dairy cows. J. Dairy Sci. 80:1076-1084.

Grings, E. E., D. M. de Avila, R. G. Eggert, and J. J. Reeves. 1990. Conception rate, growth and lactation of dairy heifers treated with recombinant somatotropin. J. Dairy Sci. 73:73-77.

Grum, D. E., J. K. Drackley, R. S. Younker, D. W. LaCount, and J. J. Veenhuizen. 1996. Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79:1850-1864.

Grummer, R. R. 1993. Etiology of lipid-related metabolic disorders in periparturient dairy cows. J. Dairy Sci. 76:3882-3890.

Grummer, R. R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition cow. J. Anim. Sci. 73:2820-2833.

Grummer, R. R., and D. J. Carroll. 1991. Effects of dietary fat on metabolic disorders and reproductive performance of dairy cattle. J. Anim. Sci. 69:3838-3852.

Grummer, R. R., P. C. Hoffman, M. L. Luck, and S. J. Bertics. 1995. Effect of prepartum and postpartum dietary energy on growth and lactation of primiparous cows. J. Dairy Sci. 78:172-180.

Grummer, R. R., K. Slark, S. J. Bertics, M. L. Luck, and J. A. Barmore. 1996. Soybeans versus animal sources of rumen-undegradable protein and fat for early lactation dairy cows. J. Dairy Sci. 79:1809-1817.

Hadsell, D. L., P. G. Campbell, and C. R. Baumrucker. 1993. Characterization of the change in type I and II insulin-like growth factor receptors of bovine mammary tissue during the pre- and postpartum periods. Endocrinology 126:637-643.

Hall, J. B., B. L. Woods, K. K. Schillo, and N. W. Bradley. 1992. Effects of prepubertal energy intake and recombinant bovine somatotropin (bST) on performance of beef heifers and their offspring. J. Anim. Sci. 70 (suppl. 1):288 (Abstr.).

Harmon, D. L. 1992. Impact of nutrition on pancreatic exocrine and endocrine secretions in ruminants. A review. J. Anim. Sci. 70:1290-1306.

Harrison, J. H., R. L. Kincaid, J. P. McNamara, S. Waltner, K. A. Loney, R. E. Riley, and J. D. Conrath. 1995. Effect of whole cottonseeds and calcium salts of long-chain fatty acids on performance of lactating dairy cows. J. Dairy Sci. 78:181-193.

Harrison R. D., I. P. Reynolds, and W. Little. 1983. A quantitative analysis of mammary glands of dairy heifers reared at different rates of live weight gain. J. Dairy Res. 50:405-412.

Hart, I. C., J. A. Bines, S. V. Morant, and J. L. Ridley. 1978. Endocrine control of energy metabolism in the cow: comparison of the levels of hormones (prolactin, growth hormone, insulin and thyroxine) and metabolites in the plasma of high-and low-yielding cattle at various stages of lactation. J. Endocrinol. 77:333-345.

Hartnell, G. F., S. E. Franson, D. E. Bauman, H. H. Head, J. T. Huber, R. C. Lamb, K. S. Madden, W. J. Cole, and R. L. Hintz. 1991. Evaluation of sometribove in a prolonged-release system in lactating dairy cows. production responses. J. Dairy Sci. 74:2645-2663.

Head, H. H., K. C.. Bachman, M. Campos, and C. J. Wilcox. 1991. Effects of plane of nutrition and somatotropin on growth rates and first lactation milk yields of Holstein heifers. J. Dairy Sci. 74(Suppl. 1):189 (Abstr.).

Head, H. H., M. Ventura, D. W. Webb, and C. J. Wilcox. 1970. Effect of growth hormone on glucose, nonesterified fatty acid and insulin levels, and on glucose utilization in dairy calves. J. Dairy Sci. 53:1496-1501.

Heinrichs, A. J., and G. L. Hargrove. 1987. Standards of weight and height for Holstein heifers. J. Dairy Sci. 70:653-660.

Heinrichs, A. J., and G. L. Hargrove. 1991. Standards of weight and height for Guernsey and Jersey heifers. J. Dairy Sci. 74:1684-1691.

Heinrichs, A. J., and G. L. Hargrove. 1994. Standards of weight and height for Ayrshire, Brown Swiss, and Milking Shorthorn heifers. J. Dairy Sci. 77:1676-1681.

Hemken, R.W., R. J. Harmon, W. J. Silvia, W. B. Tucker, G. Heersche, and R. G. Eggert. 1991. Effect of dietary energy and previous bovine somatotropin on milk yield, mastitis, and reproduction in dairy cows. J. Dairy Sci. 74:4265-4272.

Hernandez-Urdaneta, A., C. E. Coppock, R. E. McDowell, D. Gianola, and N. E. Smith. 1976. Changes in forage-concentrate ratio of complete feeds for dairy cows. J. Dairy Sci. 59:665-672.

Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836-845.

Hoffman, P. C., N. M. Brehm, S. G. Price, and A. Prill-Adams. 1996. Effect of accelerated postpubertal growth and early calving on lactation performance of primiparous Holstein heifers. J. Dairy Sci. 79:2024-2031.

Hoffman, P. C., and D. A. Funk. 1991. Growth rates of Holstein heifers in selected Wisconsin dairy herds. J. Dairy Sci. 74(Suppl. 1):212.(Abstr.).

Hoffman, P. C., and D. A. Funk. 1992. Applied dynamics of dairy replacement growth and management. J. Dairy Sci. 75:2504-2521.

Hoffman, P. C., R. R. Grummer, R. D. Shaver, G. A. Broderick, and T. R. Drendel. 1991. Feeding supplemental fat and undegraded intake protein to early lactation dairy cows. J. Dairy Sci. 74:3468-3474.

Holter, J. B., H. H. Hayes, C. K. Bozak, W. E. Urban.Jr., and M. L. McGilliard. 1990. Effect of prepartum dietary energy on condition score, postpartum energy, nitrogen partitions, and lactation production responses. J. Dairy Sci. 73:3502-3511.

Igono, M. O., H. D. Johnson, B. J. Steevens, W. A. Hainen, and M. D. Shanklin. 1988. Effect of season on milk temperature, milk growth hormone, prolactin, and somatic cell counts of lactating cattle. Int. J. Biometeorol. 32:194-200.

Iliou, J. P., and Y. Demarne. 1987. Evolution of the sensitivity of isolated adipocytes of ewes to the antilipolytic action of adenosine during pregnancy and lactation. Comp. Biochem. Physiol. 86A:755-763.

Jenny, B. F., L. W. Grimes, and F. E. Pardue. 1992. Lactational response of Jersey cows to bovine somatotropin administered daily or in a sustained-release formulation. J. Dairy Sci. 75:3402-3407.

Jerred, M. J., D. J. Carroll, D. K. Combs, and R. R. Grummer. 1990. Effects of fat supplementation and immature alfalfa to concentrate ratio on nutrient utilization and lactation performance of dairy cattle. J. Dairy Sci. 73:2842-2848.

Johnson, I. D. 1988. The effect of prepubertal nutrition on lactation performance by dairy cows. In: Nutrition and lactation in the dairy cow. P. C. Garsworthy, ed. Butterworths, London, England. p.171-183.

Johnson, T. R., and D. K. Combs. 1991. Effects of prepartum diet, inert rumen bulk, and dietary polyethylene glycol on dry matter intake of lactating dairy cows. J. Dairy Sci. 74:933-938.

Johnson, I. D., D. J. Hathom, R. M. Wilde, T. T. Treacher, and B. W. Butler-Hogg. 1987. The effects of dose and method of administration of biosynthetic bovine somatotropin. Anim. Prod. 44:405-409.

Johnson, D. G., and D. E. Otterby. 1981. Influence of dry period diet on early postpartum health, feed intake, milk production and reproductive efficiency of Holstein cows. J. Dairy Sci. 64:290-298.

Jones, J. I., and D. R. Clemmons. 1995. Insulin-like growth factors and their binding proteins: biological actions. Endocrine Rev. 6:3-34.

Journet, M., and B. Remond. 1976. Physiological factors affecting the voluntary intake of feed by cows: a review. Livest. Prod. Sci. 3:129-143.

Keown, J. F. 1986. Freshen heifers at 1200 lb. Dairy herd management, August. p. 18.

Keown, J. F., and R. W. Everett. 1986. Effects of days carried, calf, days dry, and weight of first calf heifers on yield. J. Dairy Sci. 69:1891-1897.

Kertz, A. F., L. R. Prewitt, and J. M. Ballam. 1987. Increased weight gain and effects on growth parameters of Holstein heifer calves from 3 to 12 months of age. J. Dairy Sci. 70:1612-1620.

Kertz, A. F., L. F. Reutzel, and G. M. Thomson. 1991. Dry matter intake from parturition to mid lactation. J. Dairy Sci. 74:2290-2295.

Ketelaars, J. J. M. H., and B. J. Tolkamp. 1992. Toward a new theory of feed intake regulation in ruminants. 1. Causes of differences in voluntary feed intake: critique of current views. Livest. Prod. Sci. 30:269-278.

Knickerbocker, J. J., W. W. Thatcher, F. W. Bazer, M. Drost, D. H. Barron, K. B. Fincher, and R. M. Roberts. 1986. Proteins secreted by day 16 to 18 conceptuses extend corpus luteum function in cows. J. Reprod. Fertil. 17:381-391.

Knight, C. H., and C. J. Wilde. 1987. Mammary growth during lactation: implications for increasing milk yield. J. Dairy Sci. 70:1991-1996.

Komaragiri, M. V. S., and R. A. Erdman. 1997. Factors affecting body tissue mobilization in early lactation dairy cows. 1. Effect of dietary protein on mobilization of body fat and protein. J. Dairy Sci. 80:929-937.

Komaragiri, M. V. S., R. A. Erdman, B. K. Sharma, and M. J. VandeHaar. 1994. Effect of diet undegraded intake protein (UIP) on plasma concentration of growth hormone (GH), insulin like growth factor-1 (IGF-1), and insulin like growth factor binding proteins (IGFBP's) in early lactation dairy cows. J. Dairy Sci. 77 (Suppl.1):183 (Abstr.).

Kunz, P. L., J. W. Blum, I. C. Hart, H. Bickel, and J. Landis. 1985. Effects of different energy intakes before and after calving on food intake, performance and blood hormones and metabolites in dairy cows. Anim. Prod. 40:219-225.

Lammoglia, M. A., S. T. Willard, D. M. Hallford, and R. D. Randel. 1997. Effects of dietary fat on follicular development and circulating concentrations of lipids, insulin, progesterone, estradiol-17β,13,14-dihydro-15-keto-prostaglandin F2 alpha 1 and growth hormone in estrous cyclic Brahman cows. J. Anim. Sci. 75:1591-1600.

Lanna, D. P. D., K. L. Houseknecht, D. M. Harris, and D. E. Bauman. 1995. Effect of somatotropin treatment on lipogenesis, lipolysis, and related cellular mechanisms in adipose tissue of lactating cows. J. Dairy Sci. 78:1703-1712.

Lean, I. J., H. F. Troutt, M. L. Bruss, T. B. Farver, R. L. Baldwin, J. G. Galland, D. Kratzer, C. A. Holmberg, and L. D. Weaver. 1991. Postparturient metabolic and production responses in cows previously exposed to long-term treatment with somatotropin. J. Dairy Sci. 74:3429-3445.

Lee, C. Y., H. H. Head, C. R. Feinstein, J. Hayen, and F. A. Simmen. 1995. Endocrine changes and circulating insulin-like growth factors in newborn calves fed colostrum, milk or milk replacer. AJAS. 8:51-58.

Leonard, M., and E. Block. 1997. Effects on nutrient and hormonal profile of long-term infusions of glucose or insulin plus glucose in cows treated with recombinant bovine somatotropin before peak milk yield. J. Dairy Sci. 80:127-143.

Lin, C. Y., A. J. McAllister, T. R. Batra, A. J. Lee, G. L. Roy, J. A. Vesely, and J. M. Wauthy. 1986. Production and reproduction of early and late bred dairy heifers. J. Dairy Sci. 69:760-773.

Lin, C. Y., A. J. McAllister, T. R. Batra, A. J. Lee, G. L. Roy, J. A. Vesely, J. M. Wauthy, and K. A. Winter. 1988. Effects of early and late breeding heifers on multiple lactation performance of dairy cows. J. Dairy. Sci. 71:2735-2741.

Lodge, G. A., L. J. Fisher, and J. R. Lessard. 1975. Influence of prepartum feed intake on performance of cows fed ad libitum during lactation. J. Dairy Sci. 58:696-709.

Lotan, E., E. Ziv, E. Levy, M. Morton, and H. Adler. 1988. Experimental manipulation of postpartum energy partition in high yielding dairy cows. Israel. J. Vet. Med. 44:159-167.

Lough, D. S., L. D. Muller, R. S. Kensinger, T. F. Sweeney, and L. C. Griel. 1988. Effect of added dietary fat and bovine somatotropin on the performance and metabolism of lactating dairy cows. J. Dairy Sci. 71:1161-1169.

Lucy, M. C., R. J. Collier, M. L. Kitchell, J. J. Dibner, S. D. Hauser, and G. G. Krivi. 1993. Immunohistochemical and nucleic acid analysis of somatotropin receptor populations in the bovine ovary. Biol. Reprod. 48:1219-1227.

Lucy, M. C., R. L. de la Sota, C. R. Staples, and W. W. Thatcher. 1993. Ovarian follicular populations in lactating dairy cows treated with recombinant bovine somatotropin (sometribove) or saline and fed diets differing in fat content and energy. J. Dairy Sci. 76:1014-1027.

Lucy M.C., J. D. Savio, L. Badinga, R. L. de la Sota, and W. W. Thatcher. 1992. Factors that affect ovarian follicular dynamics in cattle. J. Anim. Sci. 70:3615-3626.

Lucy M. C., C. R. Staples, F. M. Michel, and W. W. Thatcher. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. J. Dairy Sci. 74:473-482.

Lucy, M.C., C. R. Staples, W. W. Thatcher, P. S. Erickson, R. M. Cleale, J. L. Firkins, J. H. Clark, M. R. Murphy, and B. O. Brodie. 1992. Influence of diet composition, dry-matter intake, milk production and energy balance on time of post-partum ovulation and fertility in dairy cows. Anim. Prod. 54:323-331.

Machlin, L. J. 1972. Effect of porcine growth hormone on growth and carcass composition of the pig. J. Anim. Sci. 35:794-799.

Maes, M., J. M. Ketelslegers, and L. E. Underwood. 1983. Low plasma somatomedin-C in streptozotocin-induced diabetes mellitus: correlation with changes in somatogenic and lactogenic liver binding sites. Diabetes. 32:1060-1069.

Maes, M., L. E. Underwood, and J. M. Ketelslegers. 1983. Plasma somatomedin-C in fasted and refed rats: close relationship with changes in liver somatogenic but not lactogenic binding sites. J. Endocrinol. 97:243-252.

Malven P. V., H. H. Head, and R. J. Collier. 1987a. Secretion and mammary uptake of prolactin in dairy cows during lactogenesis. J. Dairy Sci. 70:2241-2253.

Malven, P. V., H. H. Head, R. J. Collier, and F. C. Buonomo. 1987b. Periparturient changes in secretion and mammary uptake of insulin and in concentrations of insulin and insulin-like growth factors in milk of dairy cows. J. Dairy Sci. 70:2254-2265.

Malven P.V., and J. P. McMurtry. 1973. Measurement of prolactin in milk by radioimmunoassay. J. Dairy Sci. 57:411-415.

Markus, S. B., K. M. Wittenberg, J. R. Ingalls, and M. Undi. 1996. Production responses by early lactation cows to whole sunflower seed or tallow supplementation of diet based on barley. J. Dairy Sci. 79:1817-1825.

Martin, R. A., and F. R. Ehle. 1986. Body composition of lactating and dry Holstein cows estimated by deuterium dilution. J. Dairy Sci. 69:88-98.

McCarty, K. S., and K. S. McCarty, Jr. 1974. Early mammary gland responses to hormones. J. Dairy Sci. 58:1022-1032.

McDowell, G. H. 1991. Somatotropin and endocrine regulation of metabolism during lactation. J. Dairy Sci. 74:44-62.

McGrath, M. F. 1987. A novel system for mammary epithelial cell culture. J. Dairy Sci. 70:1967-1980.

McGuffey, R. K., and J. I. D. Wilkinson. 1991. Nutritional implications of bovine somatotropin for the lactating dairy cow. J. Dairy Sci. 74:63-71.

McGuire, M. A., D. E. Bauman, M. M. Miller, and G. F. Hartnell. 1992a. Response of somatomedins (IGF-I and IGF-II) in lactating cows to variations in dietary energy and

protein and treatment with recombinant n-methionyl bovine somatotropin. J. Nutr. 122:128-138.

McGuire, M. M, J. M. Grinari, D. A. Dwyer, and D. E. Bauman. 1995. Role of insulin in the regulation of mammary synthesis of fat and protein. J. Dairy Sci. 78:816-824.

McGuire, M. A., J. L. Vicini, D. E. Bauman, and J. J. Veenhuizen. 1992b. Insulin-like growth factors and binding proteins in ruminants and their nutritional regulation. J. Anim. Sci. 70:2901-2910.

McNamara, J. P. 1995. Role and regulation of metabolism in adipose tissue during lactation. J. Nutr. Biochem. 6:120-129.

McNamara, J. P. 1988. Regulation of bovine adipose tissue metabolism lactation. 4. Dose-responsiveness to epinephrine as altered by stage of lactation. J. Dairy Sci. 71:643-649.

McNamara, J. P., and J. K. Hillers. 1986. Adaptations in lipid metabolism of bovine adipose tissue in lactogenesis and lactation. J. Lipid. Res. 27:150-157.

McShane, T. M., K. K. Schillo, J. A. Boling, N. W. Brandley, and J. B. Hall. 1989. Effects of recombinant DNA-derived somatotropin and dietary energy intake on development of beef heifers. I. Growth and puberty. J. Anim. Sci. 67:2230-2237.

Mertens, R. D. 1994. Regulation of forage intake. In: G. C. Fahey, Jr. (Ed) Forage Quality, Evaluation and Utilization. American Society of Agronomy, Madison, WI.

Miller, P. S., B. L. Reis, C. C. Calvert, E. J. DePeters, and R. L. Baldwin. 1991. Relationship of early lactation and bovine somatotropin on nutrient uptake by cow mammary glands. J. Dairy Sci. 74:3800-3807.

Moore, R. K., B. W. Kennedy, L. R. Schaffer, and J. E. Moxley. 1991. Relationships between age and body weight at calving and production in first lactation Ayrshires and Holsteins. J. Dairy Sci. 74:269-277.

Moran, C., and J. F. Roche. 1989. Puberty in heifers. A review. Anim. Reprod. Sci. 18:167-172.

Moseley, W. M., L. F. Krabill., and R. F. Olsen. 1982. Effect of bovine growth hormone administered in various patterns on nitrogen metabolism in the Holstein steer. J. Anim. Sci. 55:1062-1070.

Muir, L. A., S. Wien, P. F. Duquette, E. L. Rickes, and E. H. Cordes. 1983. Effects of exogenous growth hormone and diethylstilbestrol on growth and carcass composition of growing lambs. J. Anim. Sci. 56:1315-1324.

Murphy, K. D., H. Chester-Jones, B. A. Crooker, R. D. Appleman, J. E. Wheaton, and L. B. Hansen. 1991. Effects of selection for milk yield on growth, endocrine profiles and response to epinephrine. J. Anim. Sci. 69(Suppl.1):91(Abstr.).

National Research Council. 1988. Nutrient requirements of dairy cattle. 6th Ed. Natl. Acad. Press., Washington, DC.

National Research Council. 1989. Nutrient requirements of dairy cattle. 6th Rev. Ed. Natl. Acad. Press., Washington, DC.

Niezen, J. H., D. G. Grieve, B. W. McBride, and J. H. Burton. 1996. Effect of plane of nutrition before and after 200 kilograms of body weight on mammary development of prepubertal Holstein heifers. J. Dairy Sci. 79:1255-1260.

Nocek, J. E. 1996. Nutritional considerations for the transition cow. Proc Cornell Nutrition Conference. Ithaca, NY. p.122-137.

Nocek, J. E., J. E. English, and D. G. Braund. 1983. Effect of various forage feeding programs during the dry period on body condition and subsequent lactation health, production, and reproduction. J. Dairy Sci. 6:1108-1114.

Nocek, J. E., J. E. English, and D. G. Braund. 1986. Prepartum grain feeding and subsequent lactation forage program effects on performance of dairy cows in early lactation. J. Dairy Sci. 69:734-741.

Ottou, J. F., M. Doreau, and Y. Chilliard. 1995. Duodenal infusion of rapeseed oil in mid lactation cows. 6. interaction with niacin on dairy performance and nutritional balance. J. Dairy Sci. 78:1345-1352.

Palmquist, D. L. 1993. Meeting the energy needs of dairy cows during early lactation. In: Proc. Tri-State Dairy Nutr. Conf. p. 43. Ohio State Univ., Michigan State Univ., and Purdue Univ., Ft. Wayne. IN.

Park, C. S., G. M. Erickson, Y. J. Choi, and G. D. Marx. 1987. Effect of compensatory growth on regulation of growth and lactation: response of dairy heifers to a stair-step growth pattern. J. Anim. Sci. 64:1751-1758.

Pedron, O., F. Cheli, E. Senatore, D. Baroli, and R. Rizzi. 1993. Effect of body condition score at calving on performance, some blood parameters, and milk fatty acid composition in dairy cows. J. Dairy Sci. 76:2528-2535.

Pell, J. M., and P. C. Bates. 1987. Collagen and noncollagen protein turnover in skeletal muscle of growth hormone-treated lambs. J. Endocrinol. 115:R1 (abstr.).

Pell, J. M., J. C. Saunders, C. E. H. Stewart, and R. S. Gilmour. 1991. Growth hormone and nutritional control of hepatic concentration of messenger RNA for IGF-I. (Abstr.). *In*: 2nd. Int. Symp. Insulin-like Growth Factors/Somatomedins. p.149.

Pennington, J. A., and P. A. Malven. 1985. Prolactin in bovine milk near the time of calving and its relationship to premature induction of lactogenesis. J. Dairy Sci. 68:1116-1120.

Peri, I., A. Gertler, I. Bruckental, and H. Barash. 1993. The effect of manipulation in energy allowance during the rearing period of heifers on hormone concentrations and milk production in first lactation cows. J. Dairy Sci. 76:742-751.

Pethick, D. W., D. B. Lindsay, P. J. Barker, and A. Northrop. 1983. The metabolism of circulating non-esterified fatty acids by the whole animal, hind-limb muscle and uterus of pregnant ewes. Br. J. Nutr. 49:129-140.

Petterson, J. A., F. R. Dunshea, R. A. Ehrhardt, and A. W. Bell. 1993. Pregnancy and undernutrition alter glucose metabolic response to insulin in sheep. J. Nutr. 123:1286-1295.

Petterson, J. A., A.R. Slepetis, R. A. Ehrhardt, F. R. Dunshea, and A. W. Bell. 1994. Pregnancy but not moderate undernutrition attenuates insulin suppression of fat mobilization in sheep. J. Nutr. 124:2431-2439.

Phillips, L. S., J. B. Harp, S. Godstein, J. Klein, and C. I. Pao. 1990. Regulation and action of insulin-like growth at the cellular level. Proc. Nutr. Soc. 49:451-458.

Phillips, S., and T. G. Unterman. 1984. Somatomedin activity in disorders of nutrition and metabolism. Clin. Endocrinol. Metab. 13:145-155.

Politis, I., E. Block, and J. D. Turner. 1990. Effect of somatotropin on the plasminogen and plasmin system in the mammary gland: proposed mechanism of action for somatotropin on the mammary gland. J. Dairy Sci. 73:1494-1499.

Prosser, C. G., S. R. Davis, V. C. Farr, L. G. Moore, and P. D. Gluckman. 1994. Effects of close-arterial (external pudic) infusion of insulin-like growth factor-ll on milk yield and mammary blood flow in lactating goats. J. Endocrinol. 142:93-99.

Prosser, C. G., I. R. Fleet, and R. B. Heap. 1989. Action of IGF-I on mammary function. In: Biotechnology and growth regulation. pp.141-151. Eds. R. B. Heap. C. G. Prosser & G. E. Lamming. Butterworths. London, England.

Pullen, D.L., D.L.Palmquist, and R. S. Emery. 1989. Effects of days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72:49-55.

Purup, S., K. Sejrsen, and R. M. Akers. 1995. Effect of bovine GH and ovariectomy on mammary tissue sensitivity to IGF-I in prepubertal heifers. J. Endocrinol. 144:153-158.

Reeds, P. J., and M. L. Fiorotto. 1990. Growth in perspective. Proc. Nutr. Soc. Scientific Meeting part of 181 st. Scottish Meeting Aberdeen Univ., Sept. 26-28. 49:1128-1135.

Remond, B., M. Cisse, A. Ollier, and Y. Chilliard. 1991. Slow release somatotropin in dairy heifers and cows fed two levels of energy concentrate. 1. Performance and body condition. J. Dairy Sci. 74:1370-1381.

Ronge, H., and J. W. Blum. 1989. Insulin-like growth factor I responses to growth hormone in dry and lactating dairy cows. J. Anim. Physiol. Anim. Nutr. 62:280-290.

Ronge, H., J. Blum, C. Clement, F. Jans, H. Leuenberger, and H. Binder. 1988. Somatomedin C in dairy cows related to energy and protein supply and to milk production. Anim. Prod. 47:165-173.

Ruegg, P. L., and R. L. Milton. 1995. Body condition scores of Holstein cows on Prince Edward Island, Canada: Relationships with yield, reproductive performance, and disease. J. Dairy Sci. 78:552-564.

Salfer, J. A., J. G. Linn, D. E. Otterby, and W. P. Hansen. 1994. Effects of calcium salts of long-chain fatty acids added to a diet containing choice white grease on lactation performance. J. Dairy Sci. 77:2367-2375.

Samaras, S. E., H. D. Guthrie, J. A. Barber, and J. M. Hammond. 1993. Expression of the mRNAs for the insulin-like growth factors and their binding proteins during develpment of porcine ovarian follicles. Endocrinology 133:2395-2398.

Sandles L. D., and C. J. Peel. 1987. Growth and carcass composition of pre-pubertal dairy heifers treated with bovine growth hormone. Anim. Prod. 44:21-29.

Sandles, L. D., C. J. Peel, and P. D. Temple-Smith. 1987. Mammogenesis and first lactation milk yields of identical-twin heifers following pre-pubertal administration of bovine growth hormone. Anim. Prod. 45:349-358.

SAS User's Guide: Statistics, V.5. 1991. SAS Inst. Inc , Cary, NC.

Schillo, K. K., J. B. Hall, and S. M. Heleman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifers. J. Anim. Sci. 70:3994-4005.

Schingoethe, D.J., and D. P. Casper. 1991. Total lactational response to added fat during early lactation. J. Dairy Sci. 74:2617-2622.

Schneider, P .L., D. Sklan, D. S. Kronfeld, and W. Chalupa. 1990. Responses of dairy cows in early lactation to bovine somatotropin and ruminally inert fat. J. Dairy Sci. 73:1263-1268.

Sechen, S.J., F.R. Dunshea, and D.E. Bauman. 1990. Somatotropin in lactating cows: Effect on response to epinephrine and insulin. Am. J. Physiol. 258:E528.

Sejrsen, K. 1994. Relationships between nutrition, puberty and mammary development in cattle. Proc. Nutr. Soc. 53:103-111.

Sejrsen, K., and J. Foldager. 1992. Mammary growth and milk production capacity of replacement heifers in relation to diet energy concentration and plasma hormone levels. Acta Agric. Scand. (Sect. A). 42:99-105.

Serjrsen, K., J. Foldager, and D. E. Bauman. 1989. Subsequent milk yield of heifers treated with somatotropin during the pubertal phase of mammary development. Proc. Eur. Assoc. Anim. Prod. 1:447-456.

Sejrsen, K., J. Foldager, M. T. Sorensen, R. M. Akers, and D. E. Bauman. 1986. Effect of exogenous bovine somatotropin on pubertal mammary development in heifers. J. Dairy Sci. 69:1528-1535.

Sejrsen, K., J. T. Huber, H. A. Tucker, and R. M. Akers. 1982. Influence of nutrition on mammary development in pre- and postpubertal heifers. J. Dairy Sci. 65:793-798..

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: a review. J. Anim. Sci. 75:828-835.

Seymour, W. M., J. E. Nocek, and J. Siciliano-Jones. 1994. The effect of dietary fat level on performance of dairy cows at different stages of lactation. J. Dairy Sci. 72 (suppl. 1):344 (Abstr.).

Seymour, W. M., and C. E. Polan. 1986. Dietary energy regulation during gestation on subsequent lactational response to soybean meal or dried brewers grains. J. Dairy Sci. 69:2837-2845.

Seymour, W. M., J. H. Herbein, R. M. Akers, and C. E. Polan. 1988. Effect of prepartum energy intake and postpartum protein source on plasma somatotropin and insulin in lactating cows. J. Dairy Sci. 71:2936-2943.

Simmons, C. R., W. G. Bergen, M. J. Vandehaar, D. J. Sprecher, C. J. Sniffen, E. P. Stanisiewski, and H. A. Tucker. 1994. Protein and fat metabolism in cows given somavubove before parturition. J. Dairy Sci. 77:1835-1847.

Sinha, Y. N. 1995. Structural variants of prolactin: Occurrence and physiological significance. Endocrine Rev. 16:354-369.

Sinha, Y. N., and H. A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507-512.

Skaar, T. C., R. R. Grummer, M. R. Dentine, and R. H. Stauffacher. 1989. Seasonal effects of prepartum and postpartum fat and niacin feeding on lactation performance and lipid metabolism. J. Dairy Sci. 72:2028-2034.

Soderholm, C. G., D. E. Otterby, J. G. Hansen, and R. J. Annexstad. 1988. Effects of recombinant bovine somatotropin on milk production, body composition, and physiological parameters. J. Dairy Sci. 71:355-365.

Soeldner, J. S., and D. Sloane. 1965. Critical variable in the radioimmunoassay of serum insulin using the double antibody technique. Diabetes 14:771-779.

Spicer, L. J., and S. E. Echternkamp. 1995. The ovarian insulin and insulin-like growth factor system with an emphasis on domestic animals. Dom. Anim. Endocrinol. 12:223-245.

Spicer, L. J., W. B. Tucker, and G. D. Adams. 1990. Insulin-like growth factor-1 in dairy cows: relationships among energy balance, body condition, ovarian activity, and estrous behavior. J. Dairy Sci. 73:929-937.

Spicer, L. J., R. K. Vernon, W. B. Tucker, R. P. Wettemann, J. F. Hogue, and G. D. Adams. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. J. Dairy Sci. 76:2664-26673.

Stanisiewski, E. P., L. F. Krabill, and J. W. Lauderdale. 1992. Milk yield, health, and reproduction of dairy cows given somatotropin (somavubove) beginning early postpartum. J. Dairy Sci. 75:2149-2164.

Stanley, T.A., R.A. Cochran, E.S. Vanzant, D.L. Harmon, and L.R. Corah. 1993. Periparturient changes in intake, ruminal capacity, and digestive characteristics in beef cows consuming alfalfa hay. J. Anim Sci. 71:788-795.

Staples, C. R., W. W. Thatcher, and J. H. Clark. 1990. Relationship between ovarian activity and energy status during the early postpartum period of high production dairy cows. J. Dairy Sci. 73:938-947.

Staples, C. R., and H. H. Head. 1988. Short-term administration of bovine somatotropin to lactating dairy cows in a subtropical environment. J. Dairy Sci. 71:3274-3282.

Stelwagen, K., D. G. Grieve, and B. W. McBride. 1992. Growth and subsequent lactation in primigravid Holstein heifers after prepartum Bovine Somatotropin treatment. J. Dairy Sci. 75:463-472.

Stephenson, K. A., I. J. Lean, M. L. Hyde, M. A. Curtis, J. K. Garvin, and L. B. Lowe. 1997. Effects of Monensin on the metabolism of periparturient dairy cows. J. Dairy Sci. 80:830-837.

Swanson, E. W., B. J. Bearden, E. W. Culvahouse, and J. T. Miles. 1967. Restricting growth of cattle without depressing lactation. J. Dairy Sci. 50:863-869.

Tessmann, N. J., T. R. Dhiman, J. Kleinmans, H. D. Radlocff, and L. D. Satter. 1991. Recombinant bovine somatotropin with lactating cows fed diets differing in energy density. J. Dairy Sci. 74:2633-2644.

Tomlinson, D.L., R.E. James, G. L. Bethard, and M. L. McGillard. 1997. Influence of undegradability of protein in the diet on intake, daily gain, feed efficiency, and body composition of Holstein heifers. J. Dairy Sci. 80:943-948.

Tucker, H. A. 1981. Physiological control of mammary growth, lactogenesis and lactation. J. Dairy Sci. 64:1403-1421.

Tucker, H. A. 1987. Quantitative estimates of mammary growth during various physiological states: A review. J. Dairy Sci. 70:1958-1966.

Turman, E. J., and F. N. Andrews. 1955. Some effects of purified anterior pituitary growth hormone in swine. J. Anim. Sci. 14:7-13.

USDA/DHIA Bull and Cow Evaluations. 1990. Animal improvement programs laboratory. Agriculture Research Center, USDA, Beltsville, MD.

Valcavi, R., M. Zini, and I. Portioli. 1992. Thyroid hormones and growth hormone secretion. J. Endocrinol. Invest. 15:313-330.

Van Amburgh, M. E., and D. M. Galton. 1994. Accelerated growth of Holstein heifers. Effects on lactation. 56th Mtg. Proc. Cornell Nutr. Conf. for Feed Manufactures. 147-154.

VandeHaar, M. J. 1998. Current concepts in feeding dairy replacements. In: Proc. 9th. Ann. Florida Ruminant Nutr. Symp. p. 14-25.

Van Saun, R.J., S.C. Idleman, and C.J. Sniffen. 1993. Effect of undegradable protein amount fed prepartum on postpartum production in first lactation Holstein cows. J. Dairy Sci. 76:236-244.

Vasquez-Añon, M.S., S. Bertics, M. Luck, and R.R. Grummer. 1994. Peripartum liver triglyceride and plasma metabolites in dairy cows. J. Dairy Sci. 77:1521-1528.

Vega, J. R., C. A. Gibson, T. C. Skaar, D. L. Hadsell, and C. R. Baumrucker. 1991. Insulin-like growth factor (IGF)-I and II, and IGF binding proteins in serum and mammary secretions during the dry period and early lactation in dairy cows. J. Anim. Sci. 69:2538-2547.

Vernon, R. G., E. Finley, and P. W. Watt. 1991. Adenosine and the control of adrenergic regulation of adipose tissue lipolysis during lactation. J. Dairy Sci. 74:695-705.

Vernon, R. G., A. Faulkner, J. W. W. Hay, D. T. Calcert, and D. J. Flint. 1990. Insulin resistance of hind-limb tissues in vivo in lactating sheep. Biochem. J. 270:283-290.

Vernon, R. G., and E. Taylor. 1988. Insulin, dexamethasone and their interactions in the control of glucose metabolism in adipose tissue from lactating and nonlactating sheep. Biochem. J. 256:509-517.

Vicini, J. L., F. C. Buonomo, J. J. Veenhuizen, M. A. Miller, D. R. Clemons, and R. J. Collier. 1991. Nutrient balance and stage of lactation affect responses on insulin, insulin-like growth factors I and II, and insulin-like growth factor-binding protein 2 to somatotropin administration in dairy cows. J. Nutr. 121:1656-1664.

Wagner, J. F., and E. L. Veenhuizen. 1978. Growth performance, carcass composition and plasma hormone levels in wether lambs when treated with growth hormone and thyroprotein. J. Anim. Sci. 47(Suppl. 1):397(Abstr.).

Waldo, D. R. 1987. Protein and energy deposition in growing Holstein heifers. Proc. 5th Int. Symp. Protein Metabolism Nutr. Wilhelm Pieck Univ., Rostock, Germany.

Waldo, D. R., A. V. Capuco, and C. E. Rexroad, Jr.. 1988. Growing dairy heifers for optimum milk production. In Proc Southwest Nutr. Management Conf., Tucson, AZ. p30.

Wallace, A. L. C., and J. M. Bassett. 1966. Effect of sheep growth hormone on plasma insulin concentration in sheep. Metabolism 15:95-107.

Wallis, M. 1988. Mechanism of action of prolactin. In: Anonymous, In Hormones and their actions. part II. Cooke, B. A., J. B. King, and V. D. Molen eds. Elsevier. Sci. Publ., Biomed. Div. p.115-126.

Waltner, S. S., J. P. MacNamara, and J. K. Hillers. 1993. Relationships of body condition score to production variables in high producing Holstein dairy cattle. J. Dairy Sci. 76:3410-3419.

Wilcox, C. J., W. W. Tahtcher, and F. G. Martin. 1990. Statistical analysis of repeated measurements in physiology experiments. In: Proc. Final Research Co-ordination Meeting of the FAO/IAEA/ARCAL III Regional Network for Improving the Reproductive Management of Meat- and Milk-producing Livestock in Latin America with the aid of Radioimmunoassay. International Atomic Energy Agency, Vienna, Austria. p. 141-155.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, F. H. Troutt, Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to select production characteristics. J. Dairy Sci. 65:495-502.

Winder, S. J., S. D. Wheatley, and I. A. Forsyth. 1992. Receptor binding of insulin-like growth factor-I to mammary microsomes from non-pregnant, pregnant and lactating sheep. J. Endocrinol. 136:297-304.

Wright, I. A., and A. J. F. Russel. 1991. Changes in the body composition of beef cattle during compensatory growth. J. Anim. Sci. 52:105-115.

Wynn, P. C., A. L. C. Wallace, and E. F. Annison. 1979. Effects of growth hormone on wool growth and nitrogen balance in ewes. Proc. Nutr. Soc. Australia. 4:133(Abstr.).

Zamet, C. N., V. F. Colenbrander, C. J. Callahan, B. P. Chew, R. E. Erb, and N. J. Moeller. 1979. Variables associated with peripartum traits in dairy cows. II. Interrelationships among disorders and their effects on intake of feed and on reproductive efficiency. Theriogenology. 11:245-257.

Zhao, X., L. M. McBride, L. M. Trouten-Radford, L. Golfman, and J. H. Burton. 1994. Somatotropin and insulin-like growth factor-I concentrations in plasma and milk after daily or sustained release exogenous somatotropin administration. Domest. Anim. Endocrinol. 11:209-216.

BIOGRAPHICAL SKETCH

Alfredo N. Garcia Gavidia, a native Venezuelan, was born in Ojeda City, Maracaibo, on November 6, 1952. He grew up in that city and stayed there until July, 1971. He then moved to Maracaibo City to enroll in the College of Veterinary Medicine at the University of Zulia. In July 1976 he graduated from the College of Veterinary Medicine and started working in extension with dairy farmers around the Lake Maracaibo basin. In May 1977 he was admitted as a first level instructor at the College of Veterinary Medicine of the University of Zulia. In October 1978 he was promoted to the second level of instructors. In September 1980 he obtained the position of assistant professor. In January 1981 he won a scholarship from FAO to go to Argentina and enroll in the dairy cattle extension program of INTA for a 1 year period. In October 1982 he married Elsa Mattos and in January 1984 his first son Abraham was born. In April 1985 he was promoted to associate professor at the same University. During May 1986 Manuel, his second son, was born. In February 1990 Indira, his first and only daughter was born and in December of the same year he was promoted to Titular professor. In January 1992 he won a scholarship to enroll in post graduate programs (MS and Ph.D.). In August 1992 he started as a graduate student in the Department of Dairy and Poultry Sciences at the University of Florida and in December 1995 received an MS degree from this University.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

H. Herbert Head, Chairman
Professor of Dairy and Poultry Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles J. Wilcox
Professor of Dairy and Poultry Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

John E. Moore
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Douglas B. Bates
Associate Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ramon C. Littell
Professor of Statistics

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1998

Dean, College of Agriculture

Dean, Graduate School